Assessing the Relationship of White Matter Hyperintensities and Grey Matter to Neuropsychiatric Symptoms in Alzheimer’s Disease and Mild Cognitive Impairment

by

Karen Mary Misquitta

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

© Copyright by Karen Misquitta 2017
Assessing the Relationship of White Matter Hyperintensities and Grey Matter to Neuropsychiatric Symptoms in Alzheimer’s Disease and Mild Cognitive Impairment

Karen Misquitta
Master of Science
Institute of Medical Science
University of Toronto
2017

Abstract

Neuropsychiatric symptoms (NPS), including apathy, irritability and depression, are frequently encountered in patients with Alzheimer’s disease (AD). Focal brain lesions, particularly in the frontotemporal regions, have been linked to the development of NPS. Cerebrovascular disease (CVD) can cause focal lesions and is common among patients with AD. CVD can be detected on MRI as white matter hyperintensities (WMH). The current study aimed to evaluate WMH burden and regional cortical atrophy in MCI and AD and determine their relationship with NPS. WMH and grey matter lobar volumes were measured and NPS were assessed using the Neuropsychiatric Inventory. We found lower frontal, temporal and parietal GM volume and higher frontal WMH volume to be associated with NPS, with GM atrophy associated with symptom progression over 2 years. This study could provide a better understanding of the pathophysiology of NPS in AD and other dementias.
Acknowledgements

To my supervisor, Dr. Carmela Tartaglia, for your support and mentorship, and for being an incredible role model. Thank you for your trust and generosity, and for giving me the opportunity to try new things and take on new projects. I have had so many great experiences working in your lab.

To my committee members, Dr. Mary Pat McAndrews and Dr. Tomas Paus, for sharing your expertise and keen insight. Thank you also for your encouragement and sense of humour; it has been a privilege to learn from you.

To Mahsa Dadar, thank you for your patience with all my questions and for kindly sharing your knowledge and experience with me.

To Eugen Hlasny, for your kindness and wisdom.

To my fellow lab members, I am so grateful to have come to know all of you these past few years. I could never have imagined I would meet so many wonderful and talented people. Thank you for all the great memories and for your friendship, which made me look forward to coming to work every day.

Finally, to my family and friends, for your constant support throughout this process, I could not have done it without you.
Contributions

This work could not have been possible without the help of: Mahsa Dadar who provided the white matter hyperintensity segmentation volumes, pre-processed scans for the whole-brain analysis, and helped write up the methods for deformation-based morphometry and volumetric segmentation; George Tomlinson who provided statistical consultation and scripts for data analysis; and MRI and assessment data provided by the Alzheimer’s Disease Neuroimaging Initiative (ADNI).
Table of Contents

ACKNOWLEDGEMENTS ........................................................................................................................................ iii
CONTRIBUTIONS ........................................................................................................................................ iv
TABLE OF CONTENTS ...................................................................................................................................... v
LIST OF TABLES ................................................................................................................................................ ix
LIST OF FIGURES ........................................................................................................................................... x
LIST OF ABBREVIATIONS ............................................................................................................................... xi
CHAPTER 1: INTRODUCTION ........................................................................................................................ 1

1.1 Neuropsychiatric Symptoms (NPS) in dementia, Alzheimer’s Disease (AD), and mild cognitive impairment (MCI) .................................................................................................................... 1

   1.1.1 Prevalence of NPS in dementia, AD and MCI .................................................................................. 2
   1.1.1.1 MCI and NPS ......................................................................................................................... 3
   1.1.1.2 Medication use .................................................................................................................... 5
   1.1.2 NPS and AD progression ............................................................................................................ 6
   1.1.2.1 Mild behavioural impairment ............................................................................................... 8
   1.1.3 Animal models of AD ............................................................................................................... 9
   1.1.3.1 Animal models of NPS ....................................................................................................... 10
   1.1.4 Assessing neuropsychiatric symptoms in AD: The Neuropsychiatric Inventory ........... 13

1.2 AD and neuroimaging analysis .............................................................................................................. 14

   1.2.1 Brain structure and pathology in AD ........................................................................................ 15
   1.2.1.1 Amyloid plaques and neurofibrillary tangles ...................................................................... 18
   1.2.2 Neuroimaging correlates of NPS ............................................................................................... 19
   1.2.3 NPS and grey matter atrophy in AD ......................................................................................... 22

1.3 White Matter Hyperintensities and AD ............................................................................................... 23

   1.3.1 What are white matter hyperintensities (WMH)? ................................................................... 23
1.3.1.1 Risk factors of WMH ................................................................. 24
1.3.2 Pathology of WMH in AD .......................................................... 26
1.3.3 WMH and cognitive function ....................................................... 28
1.3.4 WMH and NPS ........................................................................ 30
  1.3.4.1 Vascular pathology and NPS .................................................. 32
1.4 Analysis of Brain Volume .................................................................. 33
  1.4.1 Volumetric segmentation and quantification of WMH .................. 34
1.5 Rationale, Objectives and Hypotheses ............................................. 35
  1.5.1 Hypotheses ............................................................................. 36

CHAPTER 2: MATERIALS AND METHODS ........................................ 37
2.1 Alzheimer’s Disease Neuroimaging Initiative (ADNI) ......................... 37
2.2 Subjects ....................................................................................... 37
  2.2.1 MCI and AD diagnosis ............................................................... 39
2.3 MRI ............................................................................................. 39
  2.3.1 Grey Matter Quantification ......................................................... 40
    2.3.1.1 FreeSurfer regional volumetric segmentation ....................... 40
    2.3.1.2 Deformation-based morphometry .......................................... 41
  2.3.2 WMH Quantification ................................................................. 41
2.4 NPS Assessment ........................................................................... 42
2.5 Medication Use ............................................................................ 43
2.6 Statistical Analyses ....................................................................... 44
  2.6.1 Whole-brain analysis ................................................................. 44
  2.6.2 Cross-sectional analysis ............................................................. 44
  2.6.3 Longitudinal analysis ................................................................. 45

CHAPTER 3: RESULTS ....................................................................... 48
3.1 Deformation-based morphometry ..................................................... 48
3.2 Cross-sectional analysis of GM and WMH volume to NPS........................................49
  3.2.1 WMH volume and NPS ..................................................................................50
3.3 Relative contributions of GM and WMH volumes to presence of NPS .......................57
  3.3.1 Relationship between GM and WMH volumes and NPS ..................................57
3.4 GM and WMH volume as predictors of NPS in MCI and AD ..................................63
  3.4.1 Longitudinal change in GM volume ..................................................................63
  3.4.2 Longitudinal change in WMH volume ...............................................................64
  3.4.3 GM and WMH as predictors of NPS .................................................................64
CHAPTER 4: DISCUSSION .........................................................................................69
4.1 NPS frequency in MCI and AD .............................................................................69
  4.1.1 NPS progression in MCI and AD ...................................................................72
  4.1.2 Medication use ...............................................................................................74
4.2 Identifying regions of interest associated with NPS ...........................................74
4.3 Contribution of GM and WMH volume to NPS ..................................................75
  4.3.1 Contribution of GM volume to NPS .................................................................75
    4.3.1.1 Cerebrovascular disease implications .......................................................76
  4.3.2 Contribution of WMH volume to NPS ...............................................................77
    4.3.2.1 Neurobiology of NPS in AD ..................................................................79
4.4 Clinical implications ............................................................................................80
4.5 Limitations ...........................................................................................................80
4.6 Conclusions ........................................................................................................82
4.7 Future directions ................................................................................................83
  4.7.1 Regional segmentation of GM and WMH volume ........................................83
  4.7.2 NPS and AD severity .....................................................................................84
  4.7.3 Longitudinal studies of NPS progression ......................................................85
REFERENCES ......................................................................................................87
List of Tables

Chapter 2 – Materials and Methods

Table 1: Medications used in the treatment of specific NPS .................................................................43

Chapter 3 – Results

Table 2: Demographic information for cross-sectional analysis of MCI and AD subjects ..........51
Table 3: Frequency of neuropsychiatric symptoms (NPS) in subjects with Alzheimer’s Disease (n=99) and mild cognitive impairment (n=280) .................................................................53
Table 4: Comparison of symptomatic and asymptomatic subjects with low and high WMH frontal lobe volume for NPI symptoms .....................................................................................54
Table 5: Comparison of symptomatic and asymptomatic subjects with low and high WMH temporal lobe volume for NPI symptoms .....................................................................................55
Table 6: Comparison of symptomatic and asymptomatic subjects with low and high WMH parietal lobe volume for NPI symptoms .....................................................................................56
Table 7: Ordinal logistic regressions for contribution of frontal, temporal and parietal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI and AD (n=379) subjects .....................................................................................59
Table 8: Ordinal logistic regressions for contribution of temporal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects ..........60
Table 9: Ordinal logistic regressions for contribution of parietal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects ..........61
Table 10: Ordinal logistic regressions for contribution of frontal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects ..........62
Table 11: Demographic information at baseline for longitudinal analysis of MCI and AD subjects ........................................................................................................................................65
Table 12: Frequency of neuropsychiatric symptoms (NPS) in subjects at baseline, 12 months and 24 months (n=112) ........................................................................................................66
Table 13. Frequency of neuropsychiatric symptom (NPS) subsyndromes in subjects at baseline, 12 months and 24 months (n=112) ........................................................................................................67
Table 14: Summary of generalized linear mixed model results for the frontal, temporal and parietal lobes ........................................................................................................................................68
Chapter 1 - Introduction

Figure 1: Atrophy on structural MRI scans .................................................................16
Figure 2: Model integrating Alzheimer’s disease immunohistology and biomarkers ...............17
Figure 3: Magnetic resonance images of white matter hyperintensities in two 78-year old patients .........................................................................................................................26

Chapter 2 – Materials and Methods

Figure 4: Recruitment and screening flow of MCI and AD subjects ......................................47

Chapter 3 – Results

Figure 5: Correlation coefficient maps of apathy symptoms in MCI and AD subjects ............49
Figure 6: Frequency of the frontal lobe WMH volume in MCI and AD subjects ......................51
Figure 7: Frequency of the temporal lobe WMH volume in MCI and AD subjects .................52
Figure 8: Frequency of the parietal lobe WMH volume in MCI and AD subjects ....................52

Appendix A

Figure 9: Correlation coefficient maps of agitation symptoms in MCI and AD subjects ........109
Figure 10: Correlation coefficient maps of anxiety symptoms in MCI and AD subjects ........110
Figure 11: Correlation coefficient maps of depression symptoms in MCI and AD subjects. .....111
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ</td>
<td>amyloid beta</td>
</tr>
<tr>
<td>AChEIs</td>
<td>acetylcholinesterase inhibitors</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADNI</td>
<td>Alzheimer’s Disease Neuroimaging Initiative</td>
</tr>
<tr>
<td>aMCI</td>
<td>amnestic mild cognitive impairment</td>
</tr>
<tr>
<td>na-MCI</td>
<td>non-amnestic mild cognitive impairment</td>
</tr>
<tr>
<td>APP</td>
<td>amyloid precursor protein</td>
</tr>
<tr>
<td>BEHAVE-AD</td>
<td>Behavioural Pathology in Alzheimer’s disease rating scale</td>
</tr>
<tr>
<td>CDR</td>
<td>Clinical Dementia Rating scale</td>
</tr>
<tr>
<td>CDRSB</td>
<td>Clinical Dementia Rating scale sum of boxes</td>
</tr>
<tr>
<td>CERAD-BRSD</td>
<td>Behavioural Rating Scale for Dementia from the Consortium to Establish a Registry for Alzheimer’s Disease</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CVD</td>
<td>cerebrovascular disease</td>
</tr>
<tr>
<td>DBM</td>
<td>deformation-based morphometry</td>
</tr>
<tr>
<td>DLB</td>
<td>dementia with Lewy bodies</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, 4th edition</td>
</tr>
<tr>
<td>18F-FDG-PET</td>
<td>18F-fluorodeoxyglucose positron emission tomography</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid-attenuated inversion recovery</td>
</tr>
<tr>
<td>FPCN</td>
<td>fronto-parietal control network</td>
</tr>
<tr>
<td>FTD</td>
<td>frontotemporal dementia</td>
</tr>
<tr>
<td>GM</td>
<td>grey matter</td>
</tr>
<tr>
<td>HAM-D</td>
<td>Hamilton Rating Scale for Depression</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
</tr>
<tr>
<td>HIS</td>
<td>Hachinski Ischemic Score</td>
</tr>
<tr>
<td>IADL</td>
<td>instrumental activities of daily living</td>
</tr>
<tr>
<td>MBI</td>
<td>mild behavioural impairment</td>
</tr>
<tr>
<td>MCI</td>
<td>mild cognitive impairment</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MTA</td>
<td>medial temporal lobe atrophy</td>
</tr>
<tr>
<td>mTBI</td>
<td>mild traumatic brain injury</td>
</tr>
<tr>
<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
</tr>
<tr>
<td>NPI-Q</td>
<td>Neuropsychiatric Inventory Questionnaire</td>
</tr>
<tr>
<td>NFT</td>
<td>neurofibrillary tangles</td>
</tr>
<tr>
<td>NPS</td>
<td>neuropsychiatric symptoms</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PSP</td>
<td>progressive supranuclear palsy</td>
</tr>
<tr>
<td>SPECT</td>
<td>single-photon emission computed tomography</td>
</tr>
<tr>
<td>SVD</td>
<td>small vessel disease</td>
</tr>
<tr>
<td>VaD</td>
<td>vascular dementia</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
</tr>
<tr>
<td>WMH</td>
<td>white matter hyperintensities</td>
</tr>
</tbody>
</table>
Chapter 1  Introduction

1.1 Neuropsychiatric symptoms (NPS) in dementia, Alzheimer’s disease (AD) and mild cognitive impairment (MCI)

Alzheimer’s disease (AD), the most common type of dementia, is characterized by progressive loss of cognitive function, most commonly in the memory domain. Although less well known, the majority of individuals who are diagnosed with AD also suffer from neuropsychiatric symptoms (NPS) at some point during their illness. NPS are non-cognitive disturbances that include depression, agitation, anxiety, and apathy, and can be extremely debilitating for AD patients. Studies have shown a prevalence of NPS in dementia from 50% to 80% (Lyketsos et al., 2002). NPS can reduce quality of life, lead to institutional care and contribute to caregiver burden (Kaufer et al., 1998). Moreover, neuropsychiatric symptoms can be difficult to treat (Ryu et al., 2005). Some studies also suggest that NPS may worsen cognitive symptoms and functional decline and have been associated with accelerated mortality (Alexopolous et al., 2002; Palmer et al., 2010; Rabins et al., 2006). Recent work has attempted to understand the incidence and prevalence of these symptoms during the progression of AD (Teri et al., 1999; Lyketsos et al., 2002). However, very little is known about the underlying pathophysiology of these symptoms in AD and their neuroanatomical correlates.

The pathophysiology of AD is thought to begin years before AD diagnosis. The pathological process may even begin decades before evidence of clinical impairment (Sperling et al., 2011). As a result, the National Institute on Aging and the Alzheimer’s Association (NIA-AA) divided AD into 3 phases: preclinical AD, mild cognitive impairment (MCI) due to AD, and dementia due to AD (Sperling et al., 2011). The recent treatment trial failures have resulted in a
paradigm shift whereby current trials are targeting people at asymptomatic or very mildly symptomatic stages. Aside from cognition, and with the understanding that neuropsychiatric symptoms are very common, a better understanding of neuropsychiatric symptoms might help detect people earlier in the disease course.

1.1.1 Prevalence of NPS in dementia, AD and MCI

Up to 80% of individuals with dementia are reported to experience at least one NPS since the onset of their cognitive symptoms (Lyketsos et al., 2002). Importantly, many of these symptoms tend to coexist. Lyketsos et al. (2002) found that 55% of dementia patients reported 2 or more neuropsychiatric symptoms while 44% of patients reported 3 or more. These authors identified the most frequently reported symptoms in patients with dementia to be depression, apathy and anxiety. Different neurodegenerative dementias have also appeared to show distinct neuropsychiatric features. One study compared neurobehavioral changes in AD, dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD) and discovered that patients with FTD showed significantly greater symptoms of euphoria, aberrant motor activity and disinhibition compared with AD or DLB, and those with DLB had more hallucinations (Hirono et al., 1999). The co-occurrence of apathy and depression also differs across neurodegenerative diseases. Patients diagnosed with AD, FTD, and progressive supranuclear palsy (PSP) had more severe and prevalent apathy and less depression compared with patients with Parkinson’s disease (PD) and Huntington’s disease (HD), while PD and HD had more severe and prevalent depression (Levy et al., 1998).

A recent meta-analysis by Boulay et al. (2016) reviewed the literature on NPS in AD over the past 20 years and found the most significantly reported symptoms to be apathy, depression and delusions. These results are largely corroborated by a meta-analysis by Zhao and
colleagues (2016) that also found apathy to be the most prevalent NPS in AD, followed by depression, aggression, anxiety and sleep disorders with the least common NPS being euphoria. These researchers also found that presence of delusions may be associated with age and occur more frequently in older patients. Numerous studies have noted high presence of apathy among AD subjects, ranging from 30% to 70% (Landes et al., 2001; Wetzels et al., 2010; Bruen et al., 2008). Similarly, previous studies have estimated the prevalence of depression in AD to be anywhere from 20% to 50% (Migliorelli et al., 1995; Lee et al., 2015; Lyketsos et al., 2000). Symptoms of psychosis, that include delusions and hallucinations, are often the least prevalent NPS in AD but have been associated with greater impairment (Aalten et al., 2007; Levy et al., 1996). Aalten et al. (2007) divided NPS in a group of AD subjects into hyperactivity, psychosis, affective and apathy subsyndromes. The psychosis subsyndrome, which consisted of delusions, hallucinations, and night-time behaviour disturbances, was found to be the least common (38%) but had the highest total score on the Neuropsychiatric Inventory, which measures NPS frequency and severity. A growing number of studies suggest that neuropsychiatric symptoms in dementia are clustered into subsyndromes that show similar prevalence, progression, and biological and psychosocial features across subsyndromes (Aalten et al., 2007; Robert et al., 2005).

1.1.1.1 MCI and NPS

NPS generally appear early in disease, and are often observed even at the mild cognitive impairment stage (Lyketsos et al., 2011). The concept of MCI was developed more than 20 years ago in an effort to describe the intermediate stage between healthy aging and dementia and potentially aid in early diagnosis and secondary prevention of dementia. International criteria for MCI were developed in 2003 that redefined MCI beyond cognitive impairment to include
multiple clinical profiles that could be the result of various causes (Winblad et al., 2004). The clinical criteria for MCI proposed by Albert et al. (2011) includes subjects having a change in cognition, an impairment in one or more cognitive domains, preserved independence in functional ability and being non-demented. MCI has been further classified into subcategories of amnestic MCI (aMCI), based on poor episodic memory performance on neuropsychological tests, and non-amnestic MCI (na-MCI), based on poor performance on neuropsychological tests in other cognitive domains including executive function, language and visuospatial abilities. These categories could result in impairment in either a single cognitive domain or multiple domains. Amnestic MCI is often considered a prodromal stage of AD. Not all MCI subjects will progress to dementia, and some of the cognitive deficits observed in these subjects may be due to other factors including medical comorbidities, drug use and depression (Petersen et al., 2014). The average prevalence of MCI is 18.9% of the general population according to population-based studies of MCI (Petersen et al., 2014).

Up to 50% of individuals with MCI are reported to experience at least a single NPS since cognitive symptom onset (Lyketsos et al., 2002). In one of the earliest studies to examine NPS prevalence in dementia, Lyketsos et al. (2002) found that the most frequently reported symptoms in subjects with MCI were depression, apathy and irritability. A subset of patients with MCI will go on to develop AD or other dementias and the risk of conversion to dementia is higher in MCI patients who have NPS at baseline (Rosenberg et al., 2013; Peters et al., 2013). Similarly, David et al. (2016) conducted a large 2-year prospective study to examine the trajectory of NPS using data from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). Their analysis followed 560 patients with MCI, and found that MCI subjects with worsening NPS were more likely to progress to AD and showed faster cognitive and functional decline than those whose NPS remained stable.
The majority of studies have found the prevalence of NPS to be higher in AD populations compared with MCI or cognitively healthy older adults, and higher among individuals with MCI compared with healthy populations (Geda et al., 2008; Hwang et al., 2004; Di Iulio et al., 2010). Some studies, however, found the same prevalence of NPS at baseline between individuals without cognitive impairment (26.2%) and those with MCI (28.8%) (Brodaty et al., 2012). In another study, Lyketsos et al., (2002) observed a higher prevalence of NPS in patients with dementia compared with those with MCI but found no difference in prevalence of symptoms between AD and other types of dementia, aside from a greater frequency of aberrant motor behaviour in AD.

1.1.1.2 Medication use

Medication to treat behavioural symptoms is frequently used in dementia and AD populations. Acetylcholinesterase inhibitors (AChEIs), the mainstay of symptomatic treatment in AD have not resulted in consistent results with respect to NPS. In one randomized placebo-controlled trial, AD patients taking 10mg donepezil daily had a lower Neuropsychiatric Inventory total score compared to the placebo group after 12 weeks, thereby showing improvement in their NPS (Holmes et al., 2004). On the other hand, a double-blind, placebo-controlled trial also examining the efficacy of donepezil did not find significant differences in NPS, measured by the NPI, between patients with severe AD who were randomized to donepezil compared to placebo. A systematic review of 14 randomized, placebo-controlled trials examined the effectiveness of donepezil, rivastigmine and galantamine in treating the behavioural and psychological symptoms of AD (Rodda et al., 2009). Four of the 14 studies found significant but modest differences in Neuropsychiatric Inventory total score between the drug and placebo groups. Neither has the NMDA (N-methyl-D-aspartate receptor) receptor antagonist,
memantine, resulted in clear efficacy for NPS. Some studies have shown modest benefit of memantine on improvement of neuropsychiatric symptoms such as agitation and psychosis (Hermann et al., 2011; Small & Bullock, 2011). Others, however, showed no difference in NPS between patients randomized to placebo or memantine from baseline to follow-up (Hermann et al., 2013; Fox et al., 2012). Hermann et al. (2013) conducted a randomized, double-blind placebo controlled study examining the efficacy of memantine on agitation and aggression symptoms, measured by the NPI, in moderate-to-severe AD patients. Subjects taking memantine did not differ in agitation or aggression when compared to placebo controls over 24 weeks. In addition, AChEIs and memantine do not appear to modify the progression of AD or its final outcomes (Tayeb et al., 2012). The pathophysiology of the NPS in AD is not understood, and it is unclear whether it is related to amyloid and tau pathology, therefore typical psychiatric drugs are often used. Among AD patients, it is approximated that one-third take antidepressants or antipsychotics (Gruber-Baldini et al., 2007; Kamble et al., 2009). So far, the effects of psychotropic medications, such as antidepressants and antipsychotics, have shown mixed results. While some studies observe improvement in NPS (Lyketsos et al., 2000; Rosenberg et al., 2013), other studies have reported psychotropic medications to be associated with cognitive and functional decline (Rosenberg et al., 2012) as well as an increased risk of mortality (Wang et al., 2005).

1.1.2 NPS and AD progression

As previously discussed, NPS are recognized as occurring even at the prodromal stage of AD and in those with MCI (Geda et al., 2008; Lyketsos et al., 2011). Studies have also found NPS in cognitively normal individuals to be associated with cognitive decline. For instance, longitudinal studies have found older adults with depression to have a greater risk of cognitive
decline (Geerlings et al., 2000; Wilson et al., 2002). One study examined depressive symptoms in a cohort of 7,240 older women over nearly 20 years and found a relationship between high depressive burden and cognitive decline (Zeki Al Hazzouri et al., 2014). These subjects also showed a twofold greater likelihood of developing dementia or MCI. Progression to MCI has been associated with persistent depression at baseline in cognitively normal adults (Steenland et al., 2012). Likewise, Brodaty et al. (2012) found that depression at baseline was predictive of dementia 2 years later.

An estimated 10% to 15% of individuals with MCI progress to dementia per year compared with 1% to 2% of the general population (Peterson et al., 2001; Geda et al., 2014). One study followed MCI subjects for up to 6 years and estimated the likelihood of progression to be as high as 80% (Peterson et al., 1999). A longitudinal study of MCI subjects divided NPS into stable, improved and worsened classes of NPI symptoms over time (David et al., 2016). Results showed that the worsened class had greater decline in cognitive and functional outcomes compared with the stable class. A review by Apostolova et al. (2008) found evidence that MCI patients with behavioural problems may be more likely to progress to AD. They suggested that, because amnestic MCI patients are more likely to convert to AD than nonamnestic, identifying the specific behavioural changes observed in amnestic MCI might provide prognostic information. For example, Geda et al. (2008) conducted a large study of 319 MCI subjects and 1590 cognitively normal older adults and found that apathy, agitation and irritability were higher in amnestic MCI than nonamnestic MCI while delusions and disinhibition were higher in nonamnestic MCI. They hypothesized that the symptoms prevalent in amnestic MCI may predict progression to AD, while symptoms more common to nonamnestic MCI may predict conversion to non-AD dementias. These authors also found that non-psychotic NPS, specifically apathy, agitation, anxiety, irritability and depression, affected approximately 50% of MCI individuals
and approximately 25% of cognitively normal older adults, while psychotic symptoms were rare in both MCI and normal populations. In another study, increased risk of incident dementia and AD were associated with higher scores on the Geriatric Depression Scale and the Neuropsychiatric Inventory (Rosenberg et al., 2013). Overall, these findings suggest that specific NPS may be useful as early predictors of AD or dementia.

Specific NPS have been associated with AD progression. One study followed 335 patients with incident AD (mean=3.53 years) and found psychosis and agitation/aggression predicted progression to severe dementia (Peters et al., 2015). Depression, in particular, has been linked to AD progression. According to one study, the prevalence of depression has been reported to be as high as 20% in MCI and 32% in patients with dementia (Lyketsos et al., 2002). Other studies report both presence and severity of depressive symptoms to be predictors of AD progression from MCI (Van der Mussele et al., 2014; Houde et al., 2008; Teng et al., 2007). However, Palmer et al. (2010) did not find depression symptoms or diagnosis to be associated with AD progression in amnestic MCI patients. Other studies have reported similar results (Rozzini et al., 2005; Palmer et al., 2007).

1.1.2.1 *Mild behavioural impairment*

Mild behavioural impairment (MBI) is a syndrome that generally occurs in late life and is characterized by behavioural changes and mild psychiatric symptoms but without serious cognitive complaints (Taragano et al., 2009). At a recent Alzheimer’s Association International Conference, Ismail et al. (2016) described mild behavioural impairment as a syndrome that could predict future neurodegeneration. These authors proposed a new assessment tool to measure behavioural symptoms in 5 major domains: apathy, mood, impulse control, social appropriateness and psychosis (Ismail et al., AAIC 2016). These MBI subcategories can be
related to certain NPI symptom domains such as decreased motivation (NPI: apathy/indifference); affective dysregulation (NPI: depression/dysphoria, anxiety, elation/euphoria, and irritability); impulse dyscontrol (NPI: agitation/aggression, aberrant motor behavior, and appetite/eating behavior); social inappropriateness (NPI: disinhibition); abnormal perception/thought content (NPI: delusions and hallucinations) (Ismail et al., 2016). They conducted a study evaluating 282 patients with subjective cognitive decline (SCI) or MCI using this assessment tool (Ismail et al., 2016). They found affective dysregulation to be the most frequent MBI domain (77.8%) followed by impulse dyscontrol (64.4%), decreased motivation (51.7%), social inappropriateness (27.8%) and abnormal perception/thought content (8.7%). Moreover, mean caregiver burden scores were significantly greater in MBI subjects compared to MCI. They suggested that this tool would be more appropriate for use in younger and non-demented populations than the more commonly used Neuropsychiatric Inventory Questionnaire (NPI-Q), which was designed for demented populations.

1.1.3 Animal models of AD

Animal models of dementia, including models of AD, have been developed to increase understanding of disease pathophysiology. The majority of these are mouse models that provide a means of assessing pathology in vivo and can be used to validate molecular targets and assess therapeutic compounds prior to clinical trials. They are limited, however, in their ability to fully replicate symptoms and pathogenesis. In AD, no model currently demonstrates the pathogenesis of the disease and each model may only replicate certain features of AD. Transgenic mouse models that express human amyloid precursor protein (APP) are frequently used to study AD pathology. These models mimic AB deposition, although they do not show neurofibrillary tangles or neuronal loss. The use of transgenic technology allows the creation of animal models
with lesions that are observed in human neurodegenerative diseases (Duyckaerts et al., 2008). Lesions in transgenic mouse models show clinical symptoms similar to those in humans, however these depend on the location of the lesion. Thus, an important limitation is that these are partial models of AD pathology. Other factors that may play a role in AD, such as disease comorbidities, aging and environmental factors cannot be accounted for in these models.

Both transgenic and non-transgenic mouse models of AD have been developed, and many behavioural tests, such as the radial arm water maze, the Morris water maze, and contextual fear conditioning, have been used to measure cognitive function in these mouse models (Puzzo et al., 2015). Most animal models of AD are developed from gene mutations associated with early onset familial AD, which accounts for only 1% of AD cases (Amemori et al., 2015). The majority of AD cases, however, are sporadic. Findings from animal models of this specific AD group may therefore bias interpretation of AD mechanisms overall. Identifying new genetic factors, such as that TREM2 (Triggering Receptor Expressed On Myeloid Cells 2), CD33 and CR1 (complement receptor type 1) are related to the microglial phagocytosis of Aβ (Lambert et al., 2009; Bradshaw et al., 2013; Griciuc et al., 2013), could suggest new ways of developing animal models of sporadic AD.

1.1.3.1 Animal models of NPS

Some animal models of neurodegenerative disease have also attempted to reproduce neuropsychiatric features that have been observed in individual diseases. NPS observed in mouse models of AD include locomotor activity (Dodart et al., 1999; Dumont et al., 2004; Cisse et al., 2011), anxiety (Lalonde et al., 2003, 2004; Reiserer et al., 2007), circadian disturbances (Huitron-Resendiz et al., 2002; Vloeberghs et al., 2004; Wisor et al., 2005; Ambree et al., 2006), aggression (Van Dorpe et al., 2000; Ambree et al., 2006; Vloeberghs et al., 2006; Pugh et
al., 2007) and depression (Filali et al., 2009). Increased locomotor activity, such as hyperactivity and stereotypic behaviours, show greater severity at different times of the day, and with increasing age and disease progression (Ambree et al., 2006). These may reflect fluctuations in behaviour in humans with AD commonly referred to as ‘sundowning’ where agitation and confusion may increase in the late afternoon and evening (Little et al., 1995). In addition, sleep disturbances (or circadian rhythm disturbances) are notable among AD patients. They have also been observed in mouse models where they appear to increase with disease severity (Smith, 1985; Graves et al., 2001). A majority of mouse models show anxiety disturbances (Cisse et al., 2011; Murakami et al., 2011; Filali et al., 2012), although some studies have not been able to find this association (Arendash et al., 2001b; Lalonde et al., 2002). Moreover, these symptoms can start at different ages depending on the model used (Lalonde et al., 2004; Gil-Bea et al., 2007). These behavioural disturbances are similar to neuropsychiatric symptoms of anxiety and disinhibition observed in AD patients. Aggressive behaviour has also been observed in mouse models, for instance in the form of more frequent attacks on other home cage mice and the latency to first attack when interacting with a novel mouse (Alexander et al., 2011). Aggressive behaviours tend to appear later in life in mouse models, after the development of cognitive deficits, and remain constant throughout disease progression suggesting that aggression symptoms may appear in late disease stages (Vloeberghs et al., 2006). Finally, while depressive symptoms have been observed in some mouse models (Filali et al., 2009), very few animal studies have examined these symptoms. In Filali et al. (2009), behavioural tests were used to assess irritability, apathy and depressive-like symptoms in APPswe/PS1 bigenic mouse models. On the touch escape test, where the animal’s response to the approach of an experimenter’s hand is rated, irritability to touch was found to be greater in the AD model compared to non-transgenic controls. Further, these mice showed poorer nest building and higher immobility on the Porsolt
forced swimming test suggesting greater apathy and depressive-like behaviour compared to nontransgenic controls.

A number of studies have shown neuroanatomical impairment associated with behavioural disturbances in AD mouse models. Presenilin1 and Presenilin2 double knockout mice that have age-related forebrain atrophy show certain NPS including anxiety, irritability, depression, apathy, aggressivity, anhedonia and aberrant motor behavior (Yan et al., 2013). A study by Espana et al. (2010) found accumulation of AB in the amygdala to increase fear and anxiety-like behaviour in an AD mouse model. In the TgCRND8AD mouse model of AD, regions associated with sleep-wake cycle regulation were examined (the hypothalamus, thalamus, prefrontal cortex and brainstem) (Colby-Milley et al. 2015). The highest levels of AB levels were found in the prefrontal cortex compared to the subcortical regions at 3 months, although levels in the thalamus increased by 7 months, and in the hypothalamus and brainstem by 11 months. This trajectory has been observed in human studies suggesting a role for AB accumulation in these regions with sleep-wake cycle disruption in this AD mouse model. Finally, Shruster and Offen (2014) found that targeted neurogenesis to the ventral hippocampus dentate gyrus through overexpression of Wnt3a in the 3xTgAD mouse model of AD showed decreased anxiety-related phenotype. Mouse models of NPS in AD will hopefully aid in better understanding the pathophysiology of NPS in AD and possibly ways of better targeting these symptoms.
1.1.4 Assessing neuropsychiatric symptoms in AD: The Neuropsychiatric Inventory

Various assessments have been used to measure neuropsychiatric symptoms. These scales usually measure either individual symptoms or multiple symptoms. These have included the Neuropsychiatric Inventory (NPI), the Behavioural Pathology in Alzheimer’s disease rating scale (BEHAVE-AD), the Behavioural Scale of the CERAD (CERAD-BRSD), the Brief Psychiatric Rating Scale, the Psychogeriatric Dependency Rating Scale, the Multidimensional Observation Scale for Elderly Subjects, the Revised Memory and Behavior Problems Checklist and the Geriatric Depression Scale. Despite the various study designs and neuropsychiatric assessments used, the majority of studies report similar prevalence of specific NPS. For instance, depression, agitation and apathy are among the most frequently reported symptoms in AD (Lyketsos et al., 2011; D’Onofrio et al., 2012). Among the assessments available, the NPI has been the most commonly used for measuring NPS.

The Neuropsychiatric Inventory is a structured interview used to measure psychopathology in patients with dementia (Cummings et al., 1997). It is completed by a caregiver or informant familiar with the patient. Caregivers are asked to report on the occurrence of 12 neuropsychiatric domains, which are followed by subquestions if those symptoms are present. The following 12 neuropsychiatric domains are assessed by the caregiver/informant: delusions, hallucinations, agitation or aggression, depression or dysphoria, anxiety, elation or euphoria, apathy or indifference, disinhibition, irritability or lability, motor disturbance, nighttime behaviour change, and appetite or eating change. Caregivers are then asked to report on the frequency and severity of present symptoms and rate their own level of distress for each one.
Frequency of symptoms is rated on a 4-point scale (1 = occasionally, less than once per week; 4 = very frequently, once or more per day or continuously). The severity of symptoms is scored on a 3-point scale (1=mild, 2=moderate, 3=severe). Caregiver distress is rated on a 6-point scale from 0 indicating no distress to 5 for very severe or extreme distress.

The NPI has established reliability and validity (Cummings et al., 1994, 1997). Inter-rater reliability ranged from 93.6% to 100% for the subdomains and high test-retest reliability was demonstrated for frequency (r(20)=0.79, p=0.0001) and severity (r(20)=0.86, p=0.0001) scores. Concurrent validity was also demonstrated as specific NPI subdomains were significantly correlated with domains of the BEHAVE-AD and the Hamilton Rating Scale for Depression.

Numerous studies have used the NPI to evaluate NPS in AD and in dementia populations (Geda et al., 2008; Berlow et al., 2010; Bruen et al., 2008; Lyketsos et al., 2002; Tighe et al., 2012). In a systematic review of the measures used to assess NPS in dementia, researchers highlighted the NPI due to its efficiency in examining multiple domains on a general level as well as its ability to target specific behaviours within these domains (Gitlin et al., 2014). It is also the only measure validated in multiple countries and can be used for both clinical and research purposes (Gitlin et al., 2014).

1.2 AD and neuroimaging analysis

The development of neuroimaging tools has enabled advancement in the diagnosis and understanding of neurodegenerative diseases, including AD. In addition to clinical assessment, brain imaging is used to aid in diagnosis (McKhann et al., 2011; Gorno-Tempini et al., 2011). In addition to the clinical criteria proposed by Albert et al. (2011), they propose including evidence of biomarkers, many of which are obtained through neuroimaging tools. These biomarkers
include evidence of Aβ accumulation from Positron Emission Tomography (PET) neuroimaging as well as markers of neuronal injury based on structural and functional measures, including brain atrophy and hypometabolism/hypoperfusion obtained with MRI, PET, and single-photon emission computed tomography (SPECT) imaging. Consequently, neuroimaging analysis has been shown to provide earlier diagnostic biomarkers and can help distinguish between individuals who are healthy and those with neurodegenerative disease. Neuroimaging tools can also be used to differentiate among neurodegenerative diseases including AD, dementia with Lewy bodies and frontotemporal dementia.

1.2.1 Brain structure and pathology in AD

Focal atrophy is recognized as an indicator of neurodegenerative disease and therefore volumetric analysis is frequently used to measure regions affected by disease. Changes in brain volume can occur early in Alzheimer’s disease. Atrophy generally begins years before clinical diagnosis in the anterior medial temporal lobe, characterized by smaller volumes of the hippocampus and entorhinal cortex, followed by atrophy of the lateral temporoparietal cortices (Frisoni et al., 2009; Apostolova et al., 2007). Regions typically affected in early AD have been located in the medial temporal, inferior temporal, temporal pole, inferior parietal, posterior cingulate and precuneus regions (Dickerson et al., 2009; Arnold et al., 1991; Arriagada et al., 1992). Atrophy of the amygdala (Krasuski et al., 1998), anterior parahippocampal gyrus (Krasuski et al., 1998), corpus callosum (Hampel et al., 1998; Teipel et al., 2002), and the frontal (Rusinek et al., 1991), temporal (Juottonen et al., 1998; Rusinek et al., 1991) and occipital lobes (Rusinek et al., 1991) have also been implicated in AD (figure 1). Computational methods in MRI studies have facilitated quantification of structural brain differences between groups as well as changes occurring with disease progression. While normal aging can also lead to significant
volume loss, brain imaging resolution and analysis techniques have made it easier to interpret regional brain atrophy associated with AD. Studies of the hippocampus, for instance, have shown this structure to be 15-40% smaller in AD patients (Bosscher and Scheltens 2002) and 10-15% smaller in amnestic MCI (Shi et al., 2009) compared with healthy controls. However, structural MRI is not the earliest detectable biomarker in preclinical AD; changes in tau and amyloid beta concentrations in the cerebrospinal fluid can be detected earlier during AD pathophysiology (Jack et al., 2013) (Figure 2). Nevertheless, structural MRI is an important aid for diagnosis of AD and for tracking disease progression.

Figure 1. Atrophy on structural MRI scans.

Coronal sections showing differences in brain volume in a patient with Alzheimer’s disease (A) and a healthy control subject (B). Significant atrophy can be observed in the patient with Alzheimer’s disease by visual comparison with the healthy control.
Figure 2. Model integrating Alzheimer’s disease immunohistology and biomarkers.

The threshold for biomarker detection of pathophysiological changes is denoted by the black horizontal line. The grey area denotes the zone in which abnormal pathophysiological changes lie below the biomarker detection threshold. In this figure, tau pathology precedes Aβ deposition in time, but only early on at a subthreshold biomarker detection level. Aβ deposition then occurs independently and rises above the biomarker detection threshold (purple and red arrows). This induces acceleration of tauopathy and cerebrospinal fluid (CSF) tau then rises above the detection threshold (light blue arrow). Later still, FDG PET and MRI (dark blue arrow) rise above the detection threshold. Finally, cognitive impairment becomes evident (green arrow), with a range of cognitive responses that depend on the individual’s risk profile (light green-filled area). Aβ=amyloid β. FDG=fluorodeoxyglucose. MCI=mild cognitive impairment.

Hippocampal atrophy is well established in AD research, and has been associated with clinical diagnosis of AD or MCI (Colliot et al., 2008; Jack et al., 1992), episodic memory deficits (Sarazin et al., 2010), as well as neurodegenerative pathology (Silbert et al., 2003). A growing number of studies also suggest that cortical atrophy may be a reliable measure for AD diagnosis and progression (Bakkour et al., 2013; Frisoni et al., 2009; Apostolova et al., 2007). Frisoni et al. (2009) found different patterns of cortical atrophy depending on AD severity; specifically, that
the polysynaptic hippocampal pathway (posterior cingulate/retrosplenial and medial temporal cortex) is affected in individuals with incipient AD, the direct pathway (temporal pole, temporoparietal association cortex, and dorsal prefrontal cortex) and sensorimotor and visual networks are affected in mild AD, and the sensorimotor network is affected in moderate AD.

1.2.1.1 Amyloid plaques and neurofibrillary tangles

The pattern of cortical neurodegeneration observed in imaging studies appears to model the process of neuropathological staging of neurofibrillary tangles (NFT) in AD (Braak & Braak 1995). Tau aggregates and beta-amyloid plaques are the pathological substrates of AD pathology. Amyloid plaques are the results of abnormal clusters of beta amyloid protein that build up between neurons. Tangles form due to misfolded tau protein, which aggregates within neurons, disrupting intracellular transport and leading to neuronal degeneration. While these principal pathological features of AD were identified in the upper cortical layer by Alois Alzheimer early in the 20th century, their specific role in the pathogenesis of AD remains to be understood.

Aβ deposition is believed to progress in 5 phases: 1. Aβ first deposits in the neocortex; 2. Aβ expands into allocortical brain regions; 3. Aβ then deposits in the diencephalic nuclei, the striatum, and the cholinergic nuclei of the basal forebrain; 4. Aβ can be found in several brainstem nuclei; and 5. Aβ deposits can be found in the cerebellum (Thal et al., 2002). NFT pathology appears to have a distinct progression compared to amyloid pathology. Tau aggregates tend to appear in the entorhinal region and spread to the hippocampus, amygdala and neocortex (Braak et al., 1999) and have shown correlations with deficits including memory loss (Guillozet et al., 2003). Braak and Braak (1991) defined six stages of NFT pathology describing AD progression from transentorhinal (Stage I) to entorhinal (Stage II) regions, followed by limbic
regions (Stage III, IV), neocortical sensory association and prefrontal areas (Stage V) and the
primary sensory and motor fields (Stage VI). PET imaging ligands that bind to tau or beta-
amyloid in the brain may provide an early and more accurate diagnostic biomarker for patients
with AD, and may help in the monitoring of disease progression.

Neuropathological change in AD is currently ranked based on 3 parameters, referred to as
an “ABC” score: (A) histopathological assessment of the severity of amyloid-beta plaques (B)
Braak staging of NFTs and (C) scoring of the frequency of neuritic amyloid plaques based on the
CERAD criteria (Mirra et al., 1991). There is also recognition that neuropathological changes in
AD can occur in the absence of cognitive deficits (Montine et al., 2012). Moreover, vascular and
Lewy body diseases are recognized as important co-pathologies. Other neuropathological
changes occur in AD, although these may be downstream causes of damage compared to plaques
and tangles, and include synapse loss, neuron loss, atrophy, gliosis, white matter degeneration,
granulovacuolar degeneration, and protein aggregates like Lewy bodies, TAR-DNA-binding
protein (TDP-43)–immunoreactive inclusions, actin-immunoreactive Hirano bodies, and cerebral
amyloid angiopathy (Hyman et al., 2012).

1.2.2 Neuroimaging correlates of NPS

It has long been established that underlying structural changes in the brain can contribute
to functional deficits. Previous studies have associated atrophy in specific brain regions with
cognitive and behavioural impairments; these include studies that have examined cortical
thickness, white matter tracts, grey and white matter volumes, metabolic impairment, and
perfusion studies.
There is significant heterogeneity in the literature on the neuroimaging correlates of NPS. The frontal lobes, specifically the prefrontal cortex, are frequently associated with various mental functions including executive function, attention, planning, motivation and social behaviour (Russell & Roxanas 1990). Frontal lobe regions are most frequently implicated in NPS (Boublay et al., 2016) and this has been demonstrated using structural imaging to measure differences in brain volume. For instance, Kostic et al. (2010) assessed the neuroanatomical correlates of depression in depressed vs. non-depressed Parkinson’s disease (PD) patients using voxel-based morphometry. They discovered loss of white matter in the right frontal lobe particularly in the anterior cingulate bundle and inferior orbitofrontal region. In fact, a review of NPS in PD found that frontal lobe atrophy was commonly observed in PD patients who had symptoms of depression, apathy, visual hallucinations and impulse control disorders (Alzahrani & Venneri 2015). In mTBI, severe and diffuse brain injury was considered a risk factor for psychosis especially in the frontal and temporal lobes (Zhang et al., 2003).

In addition to focal atrophy, differences in frontal lobe functional connectivity have also been associated with NPS in MCI and AD patients. Munro et al. (2015) examined the relationship between NPS and resting state functional connectivity in four brain networks (default mode network, fronto-parietal control network (FPCN), dorsal attention network, and ventral attention network) in MCI subjects. Their results showed lower FPCN connectivity related to greater affective symptoms, particularly apathy. No relationships were observed in the other networks. Recent work by Ballarini et al. (2016) examined NPS in early onset AD (<65 years of age). They divided symptoms into apathy, hyperactivity, affective and psychotic subsyndromes and looked at their relationships with metabolic dysfunction using $^{18}$F-FDG-PET. Hyperactivity and affective scores were associated with higher glucose metabolism in the frontal and limbic structures while apathy scores were negatively correlated with bilateral orbitofrontal
and dorsolateral frontal cortex metabolism. Much more remains to be understood about the role of frontal lobe structures in specific NPS, however it is likely that normal psychiatric function is dependent on the integration of multiple regions or networks (Russell & Roxanas 1990).

Symptoms of aggression have been associated with the orbitofrontal cortex, amygdala and hippocampus in human and animal studies (Coccaro et al., 2007; Soloff et al., 2003; Machado & Bachevalier 2006). Similarly, atrophy of regions of the anterior salience network, specifically frontal, insular, amygdala, cingulate and hippocampal regions, have been associated with agitation and aggression in subjects with MCI and AD (Trzepacz et al., 2013). One study using single photon emission computed tomography (SPECT) analysis in dementia patients with and without agitation/aggression symptoms measured by the NPI, found agitation/aggression to be associated with hypoperfusion of the left anterior temporal cortex, as well as the bilateral dorsofrontal and right parietal cortices (Hirono et al., 2000). Temporolimbic lesions may likewise play a role in other neuropsychiatric symptoms (Trimble et al., 1997). Research on delusions and hallucinations in patients with schizophrenia have found associations with limbic system dysfunction, particularly in the left temporal and right frontal regions and the hippocampus and amygdala (Turetsky et al., 1995; Suddath et al., 1990).

In metabolic studies of AD, regional cerebral blood flow (rCFB) deficits have been frequently reported in the temporo-parietal cortical association areas with relative sparing of subcortical grey matter structures (Smith et al., 1992; Matsuda 2001). Sultzer et al. (1995) used $^{18}$F-FDG-PET in AD patients to examine NPS assessed using the Neurobehavioral Rating Scale. They found frontal and temporal cortical hypometabolism to be associated with an agitation/disinhibition factor score, a psychosis score related to frontal cortical hypometabolism, and an anxiety/depression factor score was associated with parietal cortical hypometabolism. In
another study using PET imaging, apathy was associated with medial frontal dysfunction, specifically reduced metabolic activity in the bilateral anterior cingulate gyrus and medial orbitofrontal cortex (Marshall et al., 2007). Lower cerebral metabolism in the frontal cortex has also been observed in depressed AD subjects (Lee et al., 2006).

1.2.3 NPS and grey matter atrophy in AD

Reductions in grey matter volume of cortical and subcortical regions have been associated with specific neuropsychiatric symptoms in AD patients. A study by Bruen et al. (2008) used voxel-based morphometry (VBM) to evaluate regional grey matter volume changes associated with NPS in mild AD. They found that delusions, agitation and apathy were most related to cortical atrophy in the right hemisphere greater than the left, especially in the anterior region. Anterior right hemisphere atrophy has also been associated with delusions in other studies (Sultzer et al., 2003; Shanks and Venneri, 2004). Delusions have been associated with reduced grey matter volume of the left frontal lobe, right frontoparietal cortex and the left claustrum (Bruen et al., 2008). Irritability, anxiety and aberrant motor behaviour have been related to atrophy of the amygdala in early AD (Poulin et al., 2011). Apathy has been related to atrophy in the dorsolateral and medial prefrontal cortex, anterior cingulate areas, and the caudate and putamen (Bruen et al., 2008; Peavy et al., 2013; Rosen et al., 2005; Hahn et al., 2013; Tunnard et al., 2011; Apostolova et al., 2007; Kim et al., 2011). Donovan et al. (2014) found reduced inferior temporal cortical thickness at baseline to predict increasing apathy over a 3-year period while reduced supramarginal cortical thickness predicted increasing hallucinations.

These studies suggest that NPS, particularly depression, apathy and delusions, are most frequently associated with changes in the frontal region of the brain. A review of 118 studies over 20 years found the anterior cingulate cortex to be implicated in all 12 NPS as a result of
reduced frontal lobe volume and/or metabolic deficits (Boulay et al., 2016). Frontal lobe pathology may therefore play an important role in the neuropsychiatric symptoms observed in AD and other diseases.

1.3 White Matter Hyperintensities and AD

1.3.1 What are white matter hyperintensities (WMH)?

WMH are high signal intensity regions detected on proton density or T2-weighted MRI scans (figure 3). They can also appear as hypointense regions on T1-weighted MRI. WMH are thought to result from increased tissue water content or myelin loss and may be formed by either an increase in fluid-filled vascular spaces or gliosis (Bronge et al., 2007). These lesions serve as markers of white matter damage in the brain. The pathological substrates of WMH are heterogenous and include ischemia/hypoxia, hypoperfusion, blood-brain barrier leakage, inflammation, degeneration and amyloid angiopathy (Gouw et al., 2011).

Although the WMH are not specific, when other etiologies can be ruled out they are thought to result from small vessel disease in the brain (Pantoni & Garcia, 1997; Roman et al., 2002; Hirono et al., 2000; Erten-Lyons et al., 2013; Shim et al., 2015). These WMH are typically located in periventricular regions in the form of rims, halos, and anterior or posterior caps, and can also be found in deeper subcortical areas. They are used as a measure of cerebrovascular burden. Risk factors for developing these lesions include hypertension and atherosclerosis resulting in vascular disease (de Leeuw et al., 2000; Greenwald et al., 2001). The ischemic etiology to the WMH has been supported with repeated diffusion-weighted imaging in 5 individuals where small, clinically silent lesions were found to appear de novo in cerebral white matter over the course of 16 weekly MRI examinations (Conklin et al., 2014).
Cerebral small vessel disease is thought to cause ischemia through a number of mechanisms including vascular stenosis, occlusion of vessels, and abnormal vascular reactivity, which can consequently produce these WM changes (Wardlaw et al. 2013). Studies that have examined blood flow have found the wall of the lateral ventricle to be a region of low perfusion (Holland et al., 2008) which is also the region of high WMH volume observed on MRI. In addition, higher WMH burden in dementia patients has been associated with more severe hypoperfusion compared to the nondemented population (De Reuck et al., 1998; Hatazawa et al., 1997). Frontal lobe hypometabolism is also considered to be characteristic of subcortical small-vessel disease and WMH (Reed et al., 2004).

1.3.1.1 Risk factors of WMH

WMH also increase with age; in the general population, they are present in 11-21% of adults around age 64 and in 94% of adults around age 82 (Debette 2010). WMH, as indicators of small vessel ischemic disease, have been observed in the presymptomatic phase leading to MCI and are implicated as early predictors of MCI (Silbert et al., 2012). In addition to being associated with increasing age, vascular risk factors and MCI, previous studies have also implicated WMH in dementia (Jellinger & Attems 2005), the progression from MCI to dementia (Wolf et al., 2000), and in neuropsychological and neuropsychiatric impairment (Berlow et al., 2010; Starkstein et al., 2009; Soennesyn et al., 2012). Studies have therefore examined variability in WMH lesions rather than the presence or absence of lesions in order to compare and differentiate between healthy and disease populations.

The risk factors for cardiovascular disease, including hypertension, dyslipidemia and diabetes mellitus, have been implicated in increased risk of dementia, particularly Alzheimer’s disease and vascular dementia. For instance, longitudinal studies have linked high blood pressure
in midlife with greater risk of dementia/AD (Kivipelto et al. 2001; Korf et al. 2004). The incidence of AD has been shown to double in stroke patients (Kokmen et al., 1996) and cognitive decline following a stroke has also been observed (Andersen et al., 1996). WMH are more common in individuals with cardiovascular risk factors or cerebrovascular disease (CVD). They have also been shown to predict an increased risk of stroke, dementia and death (Debette et al., 2010). In a three-year longitudinal study, WMH progression was associated with a higher rate of conversion to dementia (Jokinen et al., 2009). These lesions may potentially serve as intermediate markers for identifying new risk factors in stroke and dementia.

WMH have also been associated with markers of endothelial dysfunction, thrombogenesis, inflammation and anti-oxidant levels (Hassan et al., 2004; Breteler et al., 1994; Schmidt et al., 1997). Genetic factors may further explain differences in WMH burden among older adults (Carmelli et al., 1998). These factors may be involved either directly with WMH or indirectly through interaction with other genes or with environmental factors. Studies suggest that WMH lesions may have a complex genetic etiology with a combination of multiple small gene effects (Assareh et al., 2011). More research is needed to explain this variability of WMH.
Figure 3. Magnetic resonance images (FLAIR) of white matter hyperintensities in two 78-year old patients.

Figure shows (A) a subject with minimal-mild white matter hyperintensity volume and (B) a subject with high white matter hyperintensity volume.

1.3.2 Pathology of WMH in AD

Research examining WMH neuropathology has used post mortem MRI and histopathology to identify the substrate of WMH. For instance, studies have linked WMH with astrogliosis, dilated perivascular spaces, arteriosclerosis and partial loss of myelin, axons and oligodendrocytes (Braffman et al., 1988; van Swieten et al., 1991; Gouw et al., 2008). Erten-Lyons and colleagues (2013) used a mixed-effects model to determine which measure of vascular pathology most strongly correlates with WMH volume accumulated over time in 66 older adults. Using MRI and histopathological data, they assessed the following measures: myelin pallor, arteriosclerosis, microvascular disease, microinfarcts, lacunar infarcts, large-vessel infarcts, atherosclerosis, neurofibrillary tangle rating, and neuritic plaque score. The most
significant contributors in their analysis were arteriosclerosis, myelin pallor and Braak score, suggesting that small vessel disease (SVD) is likely the major contributor to WMH lesions. The relationship between SVD and WMH lesions in AD has also been found in other studies (Wijesinghe et al., 2016; Shim et al., 2015). Pathogenic mechanisms that have been proposed to explain the development of WMH include cerebral amyloid angiopathy, blood brain barrier leakage, ischaemia/hypoxia, inflammation, hypoperfusion and degeneration (Gouw et al., 2011). As WMH can develop from various pathological substrates with differing severity, much remains to be understood about the specific contribution of each substrate in the pathophysiology of AD. Previous work has suggested that AD and vascular dementia (VaD) appear to show similar histopathological profiles indicating that both may share the same WMH pathogenesis (Eglund 1998). Autopsy-based studies of dementia also support the coexistence of vascular and AD pathology (Schneider et al., 2007; Olichney et al., 1997; Kalaria & Ballard, 1999). One study examined 248 autopsy cases of AD and identified 48% cerebrovascular lesions, with 31% microinfarcts, 12.5% large infarcts and 13.5% hemorrhages (Olichney et al., 1997).

While there is currently a distinction between neurodegenerative and vascular forms of dementia, studies suggest that this may be too limited a view (Mortamais et al., 2014). For instance, mixed dementia has been used to characterize individuals with both neurodegenerative and vascular pathologies. Results from the Nun Study on aging and neurodegenerative disease by Snowdon and colleagues (1997) had found that 47% of demented subjects had AD and 1 or more brain infarcts. Similarly, a more recent study showed minor and moderate vascular pathology was twice as high in AD subjects as in normal controls, particularly due to the greater frequency of acute ischemic infarcts and hemorrhages (Jellinger & Attems 2005). This suggests that a notable overlap exists and that vascular risk factors may also contribute to dementia. Cerebrovascular disease is also prevalent among AD patients, with one study even suggesting
that AD be classified as a vascular disorder (de la Torre, 2002). While an explanation for this association is currently unknown, various theories on the relationship between CVD and AD have been proposed (Mortamais et al., 2014). For instance, vascular brain injury may impair the clearance of amyloid from the brain and contribute to amyloid beta (Aβ) deposition (Hachinski et al., 1997). Alternately, Aβ deposition may lead to vascular brain injury (Han et al., 2008).

Another proposed mechanism is that ischemia could result in axonal damage and lead to retrograde degeneration (Erten-Lyons et al., 2013; Dhikav et al., 2012). Finally, vascular brain injury may act synergistically with AD pathology to lower the threshold of AD pathology required to develop AD symptoms (Tosto et al., 2014; van der Flier et al., 2004). Moreover, certain brain regions have been associated with CVD. For instance, studies have found that most WMH related to CVD are located in the frontal lobes (Mortamais et al., 2013). Significant WMH load is also found in the parietal lobes, and it has been suggested that MCI and AD may be associated with posterior WMH (Yoshita et al., 2006; Brickman et al., 2015).

1.3.3 WMH and cognitive function

Recent work has identified a relationship between WMH and cognitive impairment, although the degree of this relationship remains to be determined (Garde et al., 2000). As markers of CVD, studies on WMH have considered their role in AD and healthy older adults. Some studies have found WMH volume to be more prevalent in AD than in healthy controls (Rezek et al., 1987; Tanabe et al., 1997; Capizzano et al., 2004). For instance, Capizzano et al. (2004) identified more WMH volume in AD patients compared with healthy subjects, and also showed that WMH volume correlated with reduced cortical grey matter volume. These authors further found WMH to be associated with poorer performance on memory tasks (Capizzano et al., 2004). Gunning-Dixon and colleagues (2000) identified a relationship between WMH and
executive deficits and working memory impairment. In subjects with MCI, WMH volume was associated with a greater rate of decline in global cognition, particularly in the areas of perceptual speed, working memory, episodic memory and semantic memory even after adjusting for grey matter volume, vascular diseases and vascular risk factors (Boyle et al., 2016). The relationship between WMH and cognition was also reported in a recent meta-analysis that included 23 cross-sectional and 14 longitudinal studies (Kloppenborg et al., 2014). Likewise, a growing number of studies have identified an association between WMH lesions and cognitive decline (Habes et al., 2016; Makino et al., 2014; Benedictus et al., 2015). These researchers found a significant association between WMH and cognitive decline in all cognitive domains, particularly in general intelligence, attention and executive function. These results are corroborated by a more recent systematic review, which also found WMH volume to be associated with cognitive impairment in the general population and in individuals with MCI, memory issues or CVD (Debette et al., 2010). A number of studies suggest that WMH may have a more significant effect on executive function and information processing speed compared to memory (Schmidt et al., 2005; Prins et al., 2005). In addition, WMH lesions may have a greater effect on memory retrieval rather than encoding, as has been observed in WM disorders (Filley et al., 2001).

While some research on this topic does suggest that WMH are associated with progressive cognitive decline, not all studies have found this relationship (Tanabe et al., 1997; Mungas et al., 2002; Wahlund et al., 1996). One study that examined medial temporal lobe volume and WMH volume found only medial temporal lobe volume to be predictive of cognitive decline (Firbank et al., 2007). In middle-aged and elderly adults without disease, WMH progression over 3 years was not associated with cognitive impairment (Schmidt et al., 1998). Some randomized control trials that examined the use of antihyperintensive medication as a
means of reducing cognitive decline and dementia in older adults, revealed mixed results (Qui et al., 2005; Forette et al., 1998).

WMH may also influence functional independence in AD subjects, for example, instrumental activities of daily living (IADL) decline from aMCI to AD and with AD progression. Ogama et al. (2017) examined the frontal, temporal, parietal and occipital lobes of female subjects with AD, aMCI and normal cognition to determine the effect of regional WMH volume on the IADL. They found frontal WMH to be a predictor of impaired ability in activities of shopping and food preparation.

In summary, WMH volume was found to be high in MCI and AD patients, and has been shown to predict the progression of cognitive symptoms in AD (Brickman 2013). As a result, vascular risk factors could indirectly impact cognitive deficits associated with AD although further research in this area is required. WMH are not sufficient to predict AD and may be working in combination with other risk factors (Mortamais et al., 2014), suggesting that WMH may play a role beyond that of cognitive impairment in the AD population.

1.3.4 WMH and NPS

Cognitive impairments and neuropsychiatric symptoms appear to have distinct pathophysologies in AD (Spalletta et al., 2004; Shinno et al., 2007; Tschanz et al., 2011). Moreover, as previously discussed, atrophy alone has not been sufficient to account for NPS in AD and other dementias. Berlow et al. (2010) considered the contribution of WMH and atrophy to NPS and found that only WMH, and not whole brain or hippocampal volume, appeared to contribute to these symptoms.
Likewise, recent studies have examined WMH prevalence in AD and have found associations with NPS (Berlow et al., 2010; Starkstein et al., 2009; Soennesyn et al., 2012). In 1997, Alexopoulos and colleagues proposed the vascular depression hypothesis that associated CVD with geriatric depression. They argued that CVD predisposed, precipitated or perpetuated a depression syndrome in older adults. Since then studies have added further evidence on the prevalence of depression in AD due to white matter changes in the brain. One study by Lee et al. (2015) assessed WMH volumes of the frontal, temporal, parietal and occipital lobes and their relationship with depressive disorders (minor and major depressive disorder, dysthymic disorder and subsyndromal depression) in AD subjects. The authors found frontal lobe white matter lesion volume to be associated with depression. Moreover, Jonsson et al. (2010) found apathy to be one of the factors most consistently associated with white matter changes in dementia patients. WMH volume, especially in the frontal lobe, has been related to higher odds of apathy and higher total NPI score (Kim et al., 2013). On the other hand, Staekenborg et al. (2008) evaluated the contributions of medial temporal lobe atrophy (MTA) and WMH to behavioural and psychological symptoms assessed by the NPI and found no relationship between NPS and MTA or WMH. Measurement of medial temporal lobe atrophy and WMH were based on visual rating using the Scheltens and Fazekas scales and this method may have limited sensitivity in detecting volume changes compared with manual or automated quantification of white matter lesion volume. Periventricular WMH lesions have been associated with hallucinations, depression and anxiety in AD, particularly in regions within the frontal lobe (Soennesyn et al., 2012; Starkstein et al., 2009). Previous research in our lab has found that the frontal lobe contains a particularly high WMH load in AD, vascular dementia and mixed dementia (Anor et al., 2017). This study also found that AD patients with delusions had significantly greater WMH load compared with patients without this symptom.
Furthermore, studies identifying significant behavioural and affective changes in patients with vascular dementia (VaD) highlight the contribution of CVD to neuropsychiatric symptoms in dementia. Neuropsychiatric symptoms are often even more prevalent in VaD than in AD subjects. For instance, studies have found depression and anxiety symptoms to be more common in VaD than in AD (Padovani et al., 1995) and subjects with VaD perform worse on emotion-recognition tasks than those with AD, even with similar cognitive status (Shimokawa et al., 2000).

1.3.4.1 Vascular pathology and NPS

Various mechanisms have been proposed to explain the relationship observed between vascular pathology and NPS. Biomarkers of systemic inflammation have been associated with AD and NPS. One study suggests a vascular and inflammatory component of NPS in AD by examining the association between NPS and clinical biomarkers of cardiovascular risk and markers of systemic inflammation and microvascular pathology (Hall et al., 2013). In this study, the authors assessed NPS in patients with mild AD using the NPI, and found that 16.1% of the variance in NPS could be explained by a combination of markers, specifically Interleukin 15 (IL15), VCAM (Vascular Adhesion Molecule) and triglycerides. Nevertheless, these factors appear to account for only a small amount of variance of NPS and further investigation into these and other biomarkers are necessary.

The location of WM lesions is important when examining the underlying neuropathology associated with behavioural changes. For example, WMH pathology may contribute to disruption of WM tracts, and therefore examining WMH lesions in specific anatomic locations would be preferable compared to a more global estimation of WMH burden. For instance, Nordahl et al. (2006) examined WMH within the dorsolateral prefrontal cortex and found a
relationship between higher WMH volume in that region and decreased functional MRI activation of the dorsal prefrontal cortex.

1.4 Analysis of brain volume

MRI provides a non-invasive method of measuring brain changes associated with disease such as atrophy and cerebrovascular burden. Several neuroimaging techniques have been developed to measure volumetric changes in the brain including voxel-based morphometry (VBM), deformation-based morphometry (DBM), cortical thickness analysis, and regional volumetric segmentation tools.

VBM registers MRI images to a standard space, which allows a given coordinate to be compared among subjects as it corresponds to the same brain structure (Ashburner & Friston 2000). Similarly, DBM examines differences in vector fields that describe global or gross differences in brain shape (Ashburner et al., 1998). This technique does not segment tissue types and therefore allows a comprehensive examination of changes in brain volume. Both VBM and DBM analyses examine many brain regions simultaneously and consequently require statistical correction for multiple comparisons. These corrections may limit sensitivity to individual differences in brain structure. Cortical thickness measurement is also available from structural imaging analysis. Cortical thinning findings from neuroimaging analyses have been confirmed in post-mortem studies with changes due to neuronal loss, synaptic degeneration and cell shrinkage (Davies et al., 1987; Regeur, 2000).

Structural imaging techniques have contributed to differentiating between causes of neurodegenerative disease (Du et al., 2007) and to identifying brain-behaviour relationships. They have enabled the quantification of smaller brain structures and focal patterns of atrophy. In
addition, one study comparing imaging modalities found structural MRI to have the highest prediction accuracy (67%) of the conversion from MCI to AD (Trzepacz et al., 2014). Structural imaging is also noninvasive and is more accessible and less expensive than PET or SPECT imaging.

1.4.1 Volumetric segmentation and quantification of WMH

Volumetric segmentation is used to examine changes in volume of specific brain structures, including changes in grey matter, white matter and subcortical regions. As well, it can be used to measure areas of differing intensities such as WMH. A majority of studies have assessed the volume of WMH lesions using visual rating scales (i.e. Fazekas, Scheltens). This method, however, can lead to inconsistencies between studies and is less sensitive than volumetric assessment (van Straaten et al., 2006). Volumetric measurement of WMH lesions through manual segmentation is a more sensitive method but can be time-consuming and make it difficult to use large sample sizes. The recent development of automatic segmentation techniques of WMH has permitted large populations and samples to be studied quickly and with good reliability and validity.

Dadar and colleagues (2017) recently developed a fully automatic regression technique for WMH segmentation and volumetric quantification in aging and AD. Training data, consisting of manually labeled WMH, was used with MRI scans with multiple parameters to create a linear classifier that produces a map of binary WMH segmentations. In this study, the method was used to segment WMH in two populations: 80 subjects with normal cognition, MCI or AD from the Alzheimer’s Disease Center, and 40 healthy subjects at risk of AD from the PREVENT-AD (Pre-symptomatic evaluation of experimental or novel treatments for Alzheimer’s disease) study. The automatic regression method was also tested for performance across MRI scanners using 10
subjects from ADNI2/GO. It was found to perform well across scanners and to be an efficient and accurate segmentation technique (Dice’s Kappa = 0.62) (Dadar et al., 2017).

1.5 Rationale, Objective and Hypotheses

WMH have been shown to have a higher prevalence in AD compared with the normal aging population. Research has shown that WMH in AD are due to CVD and may be involved in AD progression. Moreover, there is growing evidence for an association between WMH and NPS. Targeting the underlying pathological substrates of WMH could therefore provide a means of preventing or treating NPS in AD patients. NPS can significantly impair quality of life, lead to medication use with potentially harmful side effects, lead to institutional care and contribute to caregiver burden. Removing or alleviating these symptoms can significantly improve quality of life for AD patients and their caregivers.

Research on WMH in AD could enable early recognition of individuals who are at risk of developing AD, even up to 10 years before the onset of MCI (Silbert et al., 2012). Early research suggests that WMH are involved in AD progression (Brickman et al., 2012; Lindemer et al., 2017). A better understanding of WMH could therefore provide a better understanding of the pathophysiology of NPS in AD and other dementias and implicate vascular disease as a therapeutic target for preventing or treating the development of WMH which, in turn, may prevent the development of NPS in AD.

This study aims to evaluate WMH volume and regional atrophy in AD and determine their relationship with NPS. This will help to better understand the neuroanatomical correlates associated with NPS in AD. This study is exploratory and does not follow a specific model with a priori hypotheses concerning the relationship between NPS, WMH and GM volumes as few
studies to date have examined this research question. As a result, we aim to identify relationships and provide insight into the neuroanatomical correlates of NPS that can aid in directing future research on these symptoms in AD and MCI subjects.

1.5.1 Hypotheses

Using all available data from ADNI 2 and ADNI GO, our hypotheses and specific aims are:

Hypothesis 1: WMH burden, especially within the frontal lobes, is related to the presence of specific NPS in MCI and AD.

Aim 1: In a cross-sectional cohort of MCI and AD subjects, we aim to evaluate WMH volume and GM volume and determine their relationship to NPS.

Hypothesis 2: Progression of WMH, particularly within the frontal lobes, and atrophy of brain regions, particularly in the frontal and temporal lobes, can predict the development of specific NPS.

Aim 2: In a longitudinal cohort of MCI and AD subjects, we aim to determine whether the appearance of WMH in specific areas and/or atrophy of specific brain regions can predict the development of specific NPS.
Chapter 2  Materials and Methods

2.1 Alzheimer’s Disease Neuroimaging Initiative (ADNI)

Participants in this study were from the Alzheimer’s Disease Neuroimaging Initiative archives. ADNI (adni.loni.usc.edu) was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The goal of ADNI is to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment and early Alzheimer’s disease. For up-to-date information, see www.adni-info.org.

Ethics approval was obtained from each study site and all research participants provided written informed consent.

2.2 Subjects

All the subjects used in this study were from the ADNI project and diagnosed with MCI or AD. In addition, ADNI selection criteria included the following: age 55-90, subjects had to have a reliable study partner to evaluate the subject’s function, had to speak English or Spanish, and have a Geriatric Depression Scale score less than 6. Subjects were also required to have a Hachinski Ischemic Score (HIS) less than or equal to 4 at baseline. The HIS is a 13-item clinical tool used to identify a vascular component in dementia patients. Patients who score 7 or higher on the scale are more likely to have vascular dementia.
ADNI exclusion criteria included any significant neurologic disease other than AD, major depression or bipolar disorder as described by the Diagnostic and Statistical manual of Mental Disorders, 4th edition, (DSM-IV) within the past year, psychotic features, agitation or behavioural problems within the last 3 months that could interfere with protocol compliance, and an MRI scan showing infection, infarction or other focal lesions at screening or baseline.

For the purpose of this study, subject had to have a NPI assessment data as well as T1-weighted and T2-weighted or proton-density (PD)-weighted MRI scans within 3 months (92 days) of their clinical assessment. Demographic data including age, sex, education and Mini-Mental State Examination (MMSE) score were also obtained from the ADNI database. All subjects were further selected based on quality control of cortical grey matter (GM) and WMH segmented MRI images. Cross-sectional analysis included 280 MCI subjects and 99 AD subjects.

The DBM analysis included 305 with MCI and 110 with AD. The higher sample size here is due to the inclusion of subjects that were removed for further analyses because of poor FreeSurfer segmentation of GM. DBM was performed to try to identify regions of interest related to NPS without a priori hypothesis.

Longitudinal analysis included 102 subjects with MCI and 10 subjects with AD, combined for analysis. Subjects for longitudinal analysis were limited to those who had data at their baseline, 12-month and 24-month visits. Six subjects with a diagnosis that was reverted from MCI to normal were removed from analysis. A breakdown of subject inclusion is provided in Figure 4. Demographic features are presented in Table 2 and Table 8.
2.2.1 MCI and AD diagnosis

Subjects recruited into ADNI as probable AD if they met the NINCDS/ADRDA criteria for probable AD with MMSE scores between 20-26 (inclusive) and a Clinical Dementia Rating scale score (CDR) of 0.5 or 1.0.

Subjects recruited into ADNI as MCI if they reported a subjective memory concern either by self-report or through an informant or clinician and had objective memory loss measured by delayed recall on the Wechsler Memory Scale Logical Memory II. MCI subjects had an MMSE score of 24-30 (inclusive) and CDR score of 0.5. Subjects must also have had preserved activities of daily living and no signs of dementia. Early and late MCI were distinguished based on education-adjusted scores from the Wechsler Memory Scale Logical Memory II (early MCI: score of 9-11 for ≥16 years of education; 5-9 for 8-15 years of education; 3-6 for 0-7 years of education; late MCI: ≤8 for ≥16 years of education; ≤4 for 8-15 years of education; ≤2 for 0-7 years of education).

2.3 MRI

T1-weighted structural MRI scans were acquired on 3 Tesla scanners (Siemens, Philips, General Electric). T1-weighted scans were used for grey matter volume segmentation and quantification. T2-weighted, proton density (PD)-weighted and fluid-attenuated inversion recovery (FLAIR) scans were collected during ADNI2/GO at 3 Tesla and used for WMH segmentation and quantification. Similar scanning protocols were used (http://adni.loni.usc.edu/methods/mri-analysis/mri-acquisition/). Scans acquired at screening/baseline, 12 months and 24 months were used for analysis. Subjects with imaging data
acquired with greater than 3 month (92 days) interval of their neuropsychological assessment were excluded from analysis.

2.3.1 Grey matter quantification

2.3.1.1 FreeSurfer regional volumetric segmentation

Grey matter cortical volumes were provided by ADNI and were segmented using FreeSurfer version 5.1 (https://surfer.nmr.mgh.harvard.edu). Frontal lobe volume was calculated by combining volumes from the following regions: superior frontal gyrus, middle frontal gyrus (rostral and caudal), inferior frontal gyrus (pars opercularis, pars triangularis, pars reticularis), orbitofrontal cortex (lateral and medial divisions), frontal pole, precentral gyrus, paracentral lobule and the anterior cingulate (rostral and caudal divisions).

Temporal lobe volume was calculated by combining volumes from the following regions: entorhinal cortex, parahippocampal gyrus, temporal pole, fusiform gyrus, temporal gyrus (superior, middle and inferior divisions), transverse temporal cortex, and banks of the temporal sulcus.

Parietal lobe volume was calculated by combining volumes from the following regions: postcentral gyrus, supramarginal gyrus, superior parietal cortex, inferior parietal cortex, precuneus cortex, posterior cingulate cortex, and isthmus cingulate cortex.

Occipital lobe volume was calculated by combining volumes from the following regions: lingual gyrus, pericalcarine cortex, cuneus cortex and the lateral occipital cortex.

Regions were chosen based on parcellations from the Desikan-Killiany atlas (Desikan et al., 2006). Volumes were corrected for total intracranial volume. Subjects were screened for
quality control of segmentations per lobe and were removed from further analysis if they failed to pass screening for the frontal, temporal and parietal lobes.

2.3.1.2  *Deformation-based morphometry*

DBM is a whole-brain analysis method that identifies macroscopic anatomical differences throughout the brain. DBM does not segment into tissue classes, and therefore provides a comprehensive and unbiased assessment of anatomical differences (Ashburner et al., 1998). In the current study, DBM was used to try to identify brain regions associated with individual NPS without any a priori hypotheses.

T1-weighted scans of the subjects were pre-processed through our standard pipeline. Image denoising (Coupe et al., 2008), intensity non-uniformity correction (Sled et al., 1998), and image intensity normalization into range (0-100) using histogram matching were performed.

DBM analysis was performed using MNI MINC tools. Pre-processed images were first linearly (using a 9-parameter rigid registration) (Collins et al., 1994) and then non-linearly warped (Collins et al., 1995) to an average template brain of 152 healthy young individuals (MNI-ICBM-152). The local deformation obtained from the non-linear transformations was used as a measure of tissue expansion or atrophy. Voxel-wise deformation maps were then corrected for age and multiple comparisons using False Discovery Rate (FDR), thresholded at 0.05.

2.3.2  WMH quantification

Manual segmentations of WMH lesions for training of the classifier were completed on T1-weighted, T2-weighted, PD-weighted and FLAIR images for 3 separate datasets: 80 AD subjects (Alzheimer’s Disease Center dataset), 40 healthy subjects at risk of AD (PREVENT-AD
dataset) and 10 subjects from ADNI2/GO. The classifier for the segmentation tool was tested on the manual segmentations from these 3 datasets (Dadar et al., 2017).

All MRI scans were pre-processed using a standardized pipeline from the MNI. Images were denoised using an automatic and multithreaded denoising method based on non-local means filtering (Manjón et al., 2010). The bias field and intensity inhomogeneity were estimated and corrected using a nonparametric non-uniform intensity normalization (N3) tool (Sled et al., 1998). The final preprocessing step included linear intensity scaling using histogram matching to a template obtained from 150 subjects (50 normal control, 50 mild cognitively impaired and 50 dementia subjects) in the ADNI database (www.loni.ucla.edu\ADNI). The T2w, PDw, and FLAIR modalities were then coregistered to the structural T1w scan of the same subject using a nine-parameter rigid body registration (Collins et al., 1994). The T1w scans were nonlinearily registered to the ADNI template based on intensity correlation coefficient (Collins and Evans, 1997). Using the T1w-to-template transformations (i.e., linear + nonlinear), the other modalities (e.g., FLAIR, T2-w, PD) were registered to the ADNI template as well. The manually segmented lesion maps were also registered to the ADNI template using the transformations of their corresponding FLAIR images.

Bilateral frontal, temporal, parietal and occipital lobe WMH volumes were segmented. Right and left volumes were combined for analysis. Few subjects had WMH volume in the occipital lobe so this region was excluded from further analysis.

2.4 NPS Assessment

Neuropsychiatric symptoms were measured using the Neuropsychiatric Inventory (NPI). The NPI is used to assess behavioural changes, principally for individuals with AD or other
dementias, which may have changed since the onset of illness. The following 12 neuropsychiatric domains are assessed by the caregiver/informant: delusions, hallucinations, agitation or aggression, depression or dysphoria, anxiety, elation or euphoria, apathy or indifference, disinhibition, irritability or lability, motor disturbance, night-time behaviour, and appetite or eating (see section 1.3 for more information about the NPI). Presence of symptoms was scored as “1” while absence of symptoms was scored as “0”. Total symptom scores, calculated by multiplying symptom frequency and severity scores, were also used for analysis.

Frequency of symptoms is rated on a 4-point scale (1 = occasionally, less than once per week; 4 = very frequently, once or more per day or continuously). The severity of symptoms is scored on a 3-point scale (1=mild, 2=moderate, 3=severe). Caregiver distress is rated on a 6-point scale from 0 indicating no distress to 5 for very severe or extreme distress.

2.5 Medication Use

Subject medication use was documented by ADNI, and medications were classified in this study as neuroleptics, antidepressants, anxiolytics, and stimulants. A summary of NPI symptoms associated with these medications are listed in Table 1 below.

Table 1. Medications used in the treatment of specific NPI symptoms

<table>
<thead>
<tr>
<th>NPI Symptom</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delusions</td>
<td>Neuroleptics</td>
</tr>
<tr>
<td>Hallucination</td>
<td>Neuroleptics</td>
</tr>
<tr>
<td>Agitation</td>
<td>Neuroleptics</td>
</tr>
<tr>
<td>Depression</td>
<td>Antidepressants</td>
</tr>
<tr>
<td>Anxiety</td>
<td>Anxiolytics</td>
</tr>
<tr>
<td>Apathy</td>
<td>Antidepressants and/or stimulants</td>
</tr>
</tbody>
</table>
2.6 Statistical Analyses

Statistical tests were performed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and R software package (version 3.3.2) (R Core Team, 2013). The Kolmogorov-Smirnov/Shapiro-Wilk tests were used to determine normality of continuous variables. Analysis of continuous variables was performed using the t-test, and the chi-square or Fisher’s exact test was used for non-continuous variables. Left and right lobar volumes of GM and WMH were combined for analysis. Results are presented as means ± standard deviation (SD). Corrections for multiple comparisons were performed for the DBM analysis but were not performed for other analyses due to the exploratory nature of this study. The significance level for all analyses was set at p<0.05.

2.6.1 Whole-brain analysis

Deformation-based morphometry (DBM) was used to identify regions associated with NPS, without any a priori hypotheses. T-tests and correlation analyses were performed on MCI (n=305), AD (n=110) and combined MCI and AD (n=415) groups independently for each symptom measured by the NPI. Results were corrected for multiple comparisons by false discovery rate, q=0.05.

2.6.2 Cross-sectional analysis

Cross-sectional analysis was conducted to test the hypothesis that WMH burden, especially within the frontal lobes, is related to the presence of specific NPS in MCI and AD. In a cross-sectional cohort of MCI and AD subjects, we evaluated WMH and GM volume and determined their relationship to NPS.
First, to examine the relationship between WMH burden and NPS, associations between lobar WMH burden and NPS were assessed. The lowest and highest tertiles of WMH volume were examined in the frontal, temporal and parietal lobes. High WMH burden was defined as above the 67th percentile of WMH volume and low WMH burden was defined as below the 33rd percentile. Difference in number of subjects from highest and lowest tertiles was assessed for each NPS using chi-square analysis. In addition, all subjects on medication relevant to specific symptoms (Table 1) were considered symptomatic of the specific neuropsychiatric symptom for which the medication is primarily indicated, i.e. depression present if on an antidepressant.

Second, ordinal logistic regressions were carried out to determine the contribution of WMH and GM volume to NPI symptom scores. Scores were grouped into absent (0), moderate (1-2) or high (3-12) symptom scores for each NPS. Frontal, temporal and parietal lobes were examined. As above, subjects on a medication used to treat specific symptoms who were scored as 0 on NPI were considered as positive for the symptom. See Table 1 for summary of medications included in analysis.

2.6.3 Longitudinal analysis

Longitudinal analysis was conducted to determine whether the progression of WMH or GM atrophy could predict the development of specific NPS. Our aim was to examine a longitudinal cohort of MCI and AD subjects to determine whether the appearance of WMH in specific areas and/or atrophy of certain brain regions can predict the development of some NPS.

Paired samples t-tests and Wilcoxon signed-rank tests were used to examine differences in GM and WMH volume between baseline, 12 months and 24 months. Generalized linear
models were used to assess the relationship between changes in GM and WMH volumes over time with presence or absence of individual NPI symptoms in the longitudinal dataset.

Subjects were grouped into 4 NPS categories: psychosis (delusions, hallucinations, sleep), apathy (apathy, appetite), hyperactivity (agitation, euphoria, disinhibition, irritability, aberrant motor behaviour) and affective (depression, anxiety) based on clusters identified by Aalten and colleagues (2007). These four neuropsychiatric subsyndromes were identified using participants collected from the European Alzheimer’s Disease Consortium, a large European database of AD patients. These subsyndromes have been used in previous studies examining NPS in AD (Zhao et al., 2016; Gonfrier et al., 2012). Frontal, temporal and parietal lobes were examined in the current study.
Subjects included in this study had a diagnosis of AD or MCI and data at their baseline, 12 months and/or 24 month visits. Subjects also had 3T MRI scans collected within 3 months of clinical assessment, as well as available NPI assessment data, FreeSurfer segmented volumes, and WMH segmented volumes. Cross-sectional and longitudinal cohorts were created for analysis. ADNI, Alzheimer’s Disease Neuroimaging Initiative; AD, Alzheimer’s disease; MCI, mild cognitive impairment; NPI, neuropsychiatric inventory; MRI, magnetic resonance imaging; WMH, white matter hyperintensities.
Chapter 3 Results

3.1 Deformation-based morphometry

DBM analysis was used to identify macroscopic differences in brain shape associated with neuropsychiatric symptoms measured by the NPI. Statistical analyses were performed on MCI (n=305), AD (n=110) and combined MCI and AD (n=415) groups for each NPI symptom. Results were corrected for age and for multiple comparisons by False Discovery Rate, q=0.05.

In addition, DBM analysis was used to correlate differences in brain volume with NPI symptom scores. No regions remained significant after FDR correction. Figure 5 shows correlation coefficient maps of regional volumes associated with NPI apathy scores in MCI and AD subjects, before FDR correction. Additional examples are provided in Appendix A.

T-tests were used to identify differences in brain structure between asymptomatic and symptomatic subjects. All 12 NPI symptoms were assessed. No significant regions were identified after FDR correction.

As a result of our lack of significant findings of an unbiased region of interest analysis using DBM, we chose to examine lobar volumes previously identified in the literature as being associated with NPS and containing regions related to NPS; specifically, we looked at the frontal, temporal and parietal lobes.
Deformation-based morphometry maps show regional volume changes associated with NPI apathy scores in MCI and AD subjects. Colours represent correlation coefficient values. Results were not significant after FDR correction (q=0.05).

3.2 Cross-sectional analysis of GM and WMH volume to NPS

Demographic details are summarized in Table 2. Of the 379 participants, 99 were AD and 280 were MCI. AD subjects were significantly older than MCI participants (p=0.004). On cognitive assessment, AD participants performed worse on the Mini Mental State Exam (MMSE) (p<0.001) and had worse scores on the CDR sum of boxes (CDRSB) (p<0.001). AD subjects also had poorer overall scores for symptom frequency and severity on the NPI (p<0.001). MCI and AD subjects did not differ significantly in sex or education level.
3.2.1 WMH volume and NPS

Analyses were conducted to assess the relationship between WMH volume and NPS. The lowest and highest tertiles of WMH volume were examined for the frontal, temporal and parietal lobes (figures 6-8). In the frontal lobe, the lowest and higher tertiles were 0-1.8 cm³ (n=123) and ≥4.1 cm³ (n=132), respectively. In the temporal lobe, the lowest and higher tertiles were 0-0.06 cm³ (n=134) and ≥0.29 cm³ (n=129), respectively. Finally, in the parietal lobe, the lowest and highest tertiles were 0-0.58 cm³ (n=125) and ≥1.49 cm³ (n=129). In addition, all subjects on medication relevant to specific symptoms (Table 1) were considered symptomatic. Results are reported at a significance of 0.05 (two-tailed) (Tables 4-6). A significantly greater number of MCI and AD patients with high parietal lobe WMH volume had irritability symptoms compared to patients with low parietal WMH volume ($\chi^2=5.237, p=0.024$).
Table 2. Demographic information for cross-sectional analysis of MCI and AD subjects

<table>
<thead>
<tr>
<th></th>
<th>MCI (n=280), mean (SD)</th>
<th>AD (n=99), mean (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.5 (7.2)</td>
<td>74.0 (7.8)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>153/127</td>
<td>55/44</td>
<td>0.907</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.4 (2.7)</td>
<td>16.1 (2.6)</td>
<td>0.329</td>
</tr>
<tr>
<td>MMSE score</td>
<td>27.9 (1.8)</td>
<td>22.9 (2.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NPI score</td>
<td>4.0 (6.3)</td>
<td>9.1 (10.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CDRSB</td>
<td>1.5 (0.9)</td>
<td>4.6 (1.7)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

AD=Alzheimer’s disease; MCI=Mild cognitive impairment; CDRSB=Clinical Dementia Rating scale, sum of boxes score; NPI=Neuropsychiatric Inventory

Figure 6. Frequency of the frontal lobe WMH volume in MCI and AD subjects.

Histogram displays white matter hyperintensity (WMH) volume derived from automatic segmentation within the frontal lobe. Most patients had low volumes.
Figure 7. Frequency of the temporal lobe WMH volume in MCI and AD subjects.

Histogram displays white matter hyperintensity (WMH) volume derived from automatic segmentation within the temporal lobe. Most patients had very low volumes.

Figure 8. Frequency of the parietal lobe WMH volume in MCI and AD subjects.

Histogram displays white matter hyperintensity (WMH) volume derived from automatic segmentation within the parietal lobe. Most patients had very low volumes.
Table 3. Frequency of neuropsychiatric symptoms (NPS) in subjects with mild cognitive impairment (n=280) and Alzheimer’s Disease (n=99)

<table>
<thead>
<tr>
<th>NPI symptom</th>
<th>All subjects (n=379)</th>
<th>MCI (n=280)</th>
<th>AD (n=99)</th>
<th>AD vs. MCI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (% of total)</td>
<td>N (% of MCI)</td>
<td>N (% of AD)</td>
<td>P</td>
</tr>
<tr>
<td>Delusions</td>
<td>19 (5.0)</td>
<td>9 (3.2)</td>
<td>10 (10.1)</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>14 (3.7) with symptoms</td>
<td>6 (2.1) with symptoms</td>
<td>8 (8.1) with symptoms</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>6 (1.6) on neuroleptics</td>
<td>3 (1.1) on neuroleptics</td>
<td>3 (3.0) on neuroleptics</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>16 (4.2)</td>
<td>7 (2.5)</td>
<td>9 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Hallucinations</td>
<td>10 (2.6) with symptoms</td>
<td>4 (1.4) with symptoms</td>
<td>6 (6.1) with symptoms</td>
<td>0.005**</td>
</tr>
<tr>
<td></td>
<td>6 (1.6) on neuroleptics</td>
<td>3 (1.1) on neuroleptics</td>
<td>3 (3.0) on neuroleptics</td>
<td>0.005**</td>
</tr>
<tr>
<td></td>
<td>77 (20.3)</td>
<td>46 (16.4)</td>
<td>31 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>74 (19.3) with symptoms</td>
<td>43 (15.4) with symptoms</td>
<td>31 (31.3) with symptoms</td>
<td>0.002**</td>
</tr>
<tr>
<td></td>
<td>6 (1.6) on neuroleptics</td>
<td>3 (1.1) on neuroleptics</td>
<td>3 (3.0) on neuroleptics</td>
<td>0.002**</td>
</tr>
<tr>
<td></td>
<td>197 (52.0)</td>
<td>136 (48.6)</td>
<td>61 (61.6)</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>113 (29.5) with symptoms</td>
<td>72 (25.7) with symptoms</td>
<td>41 (41.4) with symptoms</td>
<td>0.026*</td>
</tr>
<tr>
<td></td>
<td>136 (35.5) on antidepressants</td>
<td>101 (36.1) on antidepressants</td>
<td>34 (34.3) on antidepressants</td>
<td>0.026*</td>
</tr>
<tr>
<td></td>
<td>103 (27.2)</td>
<td>58 (20.7)</td>
<td>45 (45.5)</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>67 (17.5) with symptoms</td>
<td>39 (13.9) with symptoms</td>
<td>28 (28.3) with symptoms</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td></td>
<td>46 (12.0) on anxiolytics</td>
<td>25 (8.9) on anxiolytics</td>
<td>20 (20.2) on anxiolytics</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td></td>
<td>190 (50.1)</td>
<td>122 (43.6)</td>
<td>68 (68.7)</td>
<td></td>
</tr>
<tr>
<td>Apathy</td>
<td>90 (23.7) with symptoms</td>
<td>44 (15.7) with symptoms</td>
<td>46 (46.5) with symptoms</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td></td>
<td>135 (35.6) on antidepressants/stimulants</td>
<td>101 (36.1) on antidepressants/stimulants</td>
<td>34 (34.3) on antidepressants/stimulants</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Euphoria</td>
<td>9 (2.4)</td>
<td>7 (2.5)</td>
<td>2 (2.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>46 (12.1)</td>
<td>25 (8.9)</td>
<td>21 (21.2)</td>
<td>0.001**</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>94 (24.8)</td>
<td>60 (21.4)</td>
<td>34 (34.3)</td>
<td>0.011*</td>
</tr>
<tr>
<td>Aberrant motor behaviour</td>
<td>28 (7.4)</td>
<td>11 (3.9)</td>
<td>17 (17.2)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>69 (18.2)</td>
<td>49 (17.5)</td>
<td>20 (20.2)</td>
<td>0.549</td>
</tr>
<tr>
<td>Appetite change</td>
<td>52 (13.7)</td>
<td>29 (10.4)</td>
<td>23 (23.2)</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

NPI=Neuropsychiatric Inventory; AD=Alzheimer’s Disease; MCI=mild cognitive impairment
Significant results, *p<0.05, **p<0.01, ***p<0.001
Table 4. Comparison of symptomatic and asymptomatic subjects with low and high frontal lobe WMH volume for NPI symptoms

<table>
<thead>
<tr>
<th>NPI symptom</th>
<th>Frontal lobe WMH volume</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest tertile</td>
<td>Highest tertile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-1.8cm³</td>
<td>&gt;4.1cm³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N&lt;br&gt;_LT=123</td>
<td>N&lt;br&gt;_HT=132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
<td>χ²</td>
<td>p</td>
</tr>
<tr>
<td>Delusions</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>5.5</td>
<td>0.172</td>
<td>0.787</td>
<td></td>
</tr>
<tr>
<td>Hallucinations</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>3.9</td>
<td>1.386</td>
<td>0.337</td>
<td></td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>23</td>
<td>30</td>
<td>53</td>
<td>20.8</td>
<td>0.627</td>
<td>0.444</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>62</td>
<td>70</td>
<td>132</td>
<td>51.8</td>
<td>0.176</td>
<td>0.693</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>35</td>
<td>37</td>
<td>72</td>
<td>28.2</td>
<td>0.006</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Euphoria</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>2.4</td>
<td>0.546</td>
<td>0.685</td>
<td></td>
</tr>
<tr>
<td>Apathy</td>
<td>57</td>
<td>68</td>
<td>125</td>
<td>49.0</td>
<td>0.682</td>
<td>0.453</td>
<td></td>
</tr>
<tr>
<td>Disinhibition</td>
<td>13</td>
<td>19</td>
<td>32</td>
<td>12.5</td>
<td>0.849</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>26</td>
<td>41</td>
<td>67</td>
<td>26.3</td>
<td>3.236</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>6</td>
<td>11</td>
<td>17</td>
<td>6.7</td>
<td>1.222</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>28</td>
<td>28</td>
<td>56</td>
<td>22.0</td>
<td>0.089</td>
<td>0.880</td>
<td></td>
</tr>
<tr>
<td>Appetite change</td>
<td>19</td>
<td>18</td>
<td>37</td>
<td>14.5</td>
<td>0.168</td>
<td>0.724</td>
<td></td>
</tr>
</tbody>
</table>

NPI=Neuropsychiatric Inventory; WMH=white matter hyperintensities
Significant results, *p<0.05
Table 5. Comparison of symptomatic and asymptomatic subjects with low and high temporal lobe WMH volume for NPI symptoms

<table>
<thead>
<tr>
<th>NPI symptom</th>
<th>Temporal lobe WMH volume</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest tertile (0-0.06cm³)</td>
<td>Highest tertile (&gt;0.29cm³)</td>
<td>Total&lt;sub&gt;LT+HT&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N&lt;sub&gt;LT&lt;/sub&gt;=134</td>
<td>N&lt;sub&gt;HT&lt;/sub&gt;=129</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Delusions</td>
<td>11</td>
<td>8.2</td>
<td>3</td>
<td>2.3</td>
<td>14</td>
<td>5.3</td>
<td>4.514</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>7</td>
<td>5.2</td>
<td>2</td>
<td>1.6</td>
<td>9</td>
<td>3.4</td>
<td>2.684</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>26</td>
<td>19.4</td>
<td>23</td>
<td>17.8</td>
<td>49</td>
<td>18.6</td>
<td>0.107</td>
</tr>
<tr>
<td>Depression</td>
<td>69</td>
<td>51.5</td>
<td>75</td>
<td>58.1</td>
<td>144</td>
<td>54.8</td>
<td>1.172</td>
</tr>
<tr>
<td>Anxiety</td>
<td>32</td>
<td>23.9</td>
<td>37</td>
<td>28.7</td>
<td>69</td>
<td>26.2</td>
<td>0.783</td>
</tr>
<tr>
<td>Euphoria</td>
<td>2</td>
<td>1.5</td>
<td>5</td>
<td>3.9</td>
<td>7</td>
<td>2.7</td>
<td>1.441</td>
</tr>
<tr>
<td>Apathy</td>
<td>68</td>
<td>50.7</td>
<td>69</td>
<td>53.5</td>
<td>137</td>
<td>52.1</td>
<td>0.198</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>13</td>
<td>9.7</td>
<td>20</td>
<td>15.5</td>
<td>33</td>
<td>12.5</td>
<td>2.017</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>27</td>
<td>20.1</td>
<td>29</td>
<td>22.5</td>
<td>56</td>
<td>21.3</td>
<td>0.213</td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>8</td>
<td>6.0</td>
<td>10</td>
<td>7.8</td>
<td>18</td>
<td>6.8</td>
<td>0.327</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>21</td>
<td>15.7</td>
<td>28</td>
<td>21.7</td>
<td>49</td>
<td>18.6</td>
<td>1.578</td>
</tr>
<tr>
<td>Appetite change</td>
<td>19</td>
<td>14.2</td>
<td>14</td>
<td>10.9</td>
<td>33</td>
<td>12.5</td>
<td>0.663</td>
</tr>
</tbody>
</table>

NPI=Neuropsychiatric Inventory; WMH-white matter hyperintensities
Significant results, *p<0.05
Table 6. Comparison of symptomatic and asymptomatic subjects with low and high parietal lobe WMH volume for NPI symptoms

<table>
<thead>
<tr>
<th>NPI symptom</th>
<th>Parietal lobe WMH volume</th>
<th></th>
<th></th>
<th></th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest tertile</td>
<td>Highest tertile</td>
<td>Total LT+HT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-0.58cm³</td>
<td>&gt;1.49cm³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N&lt;sub&gt;LT&lt;/sub&gt;=125</td>
<td>N&lt;sub&gt;HT&lt;/sub&gt;=129</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Delusions</td>
<td>8</td>
<td>6.4</td>
<td>5</td>
<td>3.9</td>
<td>13</td>
<td>5.1</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>4</td>
<td>3.2</td>
<td>6</td>
<td>4.7</td>
<td>10</td>
<td>3.9</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>25</td>
<td>20.0</td>
<td>24</td>
<td>18.6</td>
<td>49</td>
<td>19.3</td>
</tr>
<tr>
<td>Depression</td>
<td>61</td>
<td>48.8</td>
<td>69</td>
<td>53.5</td>
<td>130</td>
<td>51.2</td>
</tr>
<tr>
<td>Anxiety</td>
<td>32</td>
<td>25.6</td>
<td>32</td>
<td>24.8</td>
<td>64</td>
<td>25.2</td>
</tr>
<tr>
<td>Euphoria</td>
<td>1</td>
<td>0.8</td>
<td>4</td>
<td>3.1</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td>Apathy</td>
<td>58</td>
<td>46.4</td>
<td>69</td>
<td>53.5</td>
<td>127</td>
<td>50.0</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>12</td>
<td>9.6</td>
<td>19</td>
<td>14.7</td>
<td>31</td>
<td>12.2</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>20</td>
<td>16.0</td>
<td>36</td>
<td>27.9</td>
<td>56</td>
<td>22.0</td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>10</td>
<td>8.0</td>
<td>11</td>
<td>8.5</td>
<td>21</td>
<td>8.3</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>21</td>
<td>16.8</td>
<td>25</td>
<td>19.4</td>
<td>46</td>
<td>18.1</td>
</tr>
<tr>
<td>Appetite change</td>
<td>16</td>
<td>12.8</td>
<td>20</td>
<td>15.5</td>
<td>36</td>
<td>14.2</td>
</tr>
</tbody>
</table>

NPI=Neuropsychiatric Inventory; WMH=white matter hyperintensities
Significant results, *p<0.05
3.3 Relative contributions of GM and WMH volumes to presence of NPS

A summary of the frequency of NPI symptoms in the current population is listed in table 3. The most frequent NPI symptom observed among AD subjects was apathy, followed by depression, irritability and agitation. The most frequent NPI symptom observed in the MCI population was depression, followed by irritability, apathy and agitation. MCI and AD groups differed most significantly in symptoms of anxiety, apathy and aberrant motor behaviour (p<0.001) with significantly more AD patients showing these symptoms.

3.3.1 Relationship between GM and WMH volumes and NPS

Ordinal logistic regression was used to examine the relative contribution of GM and WMH volumes to absent (score of 0), moderate (score of 1-2) and high (score of 3-12) NPI symptom scores over 2 years. Results are reported in tables 7-10. Frontal, temporal and parietal lobe volumes were examined in relation to NPI symptoms. In combined MCI and AD subjects, lower GM volume in the frontal (OR=0.361, p=0.016), temporal (OR=0.276, p=0.009) and parietal (OR=0.326, p=0.010) lobes after ICV correction was associated with hallucinations (table 7). Lower GM volume in the frontal (OR=0.626, p=0.002), temporal (OR=0.553, p=0.001) and parietal (OR=0.588, p=0.002) lobes was significantly associated with apathy. Lower GM volume in the frontal (OR=0.681, p=0.042), temporal (OR=0.639, p=0.042), and parietal (OR=0.582, p=0.010) lobes was associated with appetite change. In this group, higher WMH volume in the frontal lobe was related to irritability (OR=1.055, p=0.007).
Ordinal regressions were completed on MCI and AD groups separately to identify differences between diagnoses. Higher depression, disinhibition and sleep were associated with greater WMH volume in the frontal lobe in AD (OR=1.086, p=0.048; OR=1.095, p=0.047; and OR=1.116, p=0.022; respectively). Lower temporal lobe GM volume was associated with less disinhibition (OR=2.505, p=0.045) and lower GM volume in the parietal lobe was related to less agitation (OR=1.977, p=0.031) in AD. In MCI, higher WMH load in the frontal lobe was related to increased symptoms of irritability (OR=1.052, p=0.022).
Table 7. Ordinal logistic regressions for contribution of frontal, temporal and parietal lobe grey matter and WMH volumes to the presence of NPI symptoms in combined MCI and AD subjects (n=379).

<table>
<thead>
<tr>
<th>NPI Symptom</th>
<th>Frontal Lobe</th>
<th>Temporal Lobe</th>
<th>Parietal Lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grey matter volume (mm$^3$)</td>
<td>White matter hyperintensity volume (cm$^3$)</td>
<td>Grey matter volume (mm$^3$)</td>
</tr>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>Delusions</td>
<td>0.749 (0.382 - 1.459)</td>
<td>0.395</td>
<td>1.018 (0.912 - 0.993)</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0.361 (0.152 - 0.809)</td>
<td><strong>0.016</strong>*</td>
<td>0.999 (0.857 - 1.089)</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>0.953 (0.695 - 1.307)</td>
<td>0.765</td>
<td>1.006 (0.958 - 1.049)</td>
</tr>
<tr>
<td>Depression</td>
<td>0.969 (0.740 - 1.270)</td>
<td>0.821</td>
<td>1.013 (0.972 - 1.053)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.930 (0.672 - 1.287)</td>
<td>0.663</td>
<td>1.021 (0.972 - 1.066)</td>
</tr>
<tr>
<td>Euphoria</td>
<td>0.744 (0.323 - 1.695)</td>
<td>0.481</td>
<td>0.999 (0.843 - 1.095)</td>
</tr>
<tr>
<td>Apathy</td>
<td>0.626 (0.462 - 0.843)</td>
<td><strong>0.002</strong>*</td>
<td>0.998 (0.951 - 1.043)</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.903 (0.613 - 1.329)</td>
<td>0.605</td>
<td>1.032 (0.978 - 1.081)</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>0.764 (0.568 - 1.025)</td>
<td>0.074</td>
<td>1.055 (1.014 - 1.098)</td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>0.812 (0.502 - 1.309)</td>
<td>0.394</td>
<td>1.026 (0.956 - 1.083)</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>0.947 (0.683 - 1.313)</td>
<td>0.745</td>
<td>1.009 (0.957 - 1.056)</td>
</tr>
<tr>
<td>Appetite change</td>
<td>0.681 (0.469 - 0.983)</td>
<td><strong>0.042</strong>*</td>
<td>1.016 (0.962 - 1.066)</td>
</tr>
</tbody>
</table>

OR=odds ratio; CI=confidence Interval

*Significant results, p<0.05
Table 8. Ordinal logistic regressions for contribution of frontal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects separately.

<table>
<thead>
<tr>
<th>NPI Symptom</th>
<th>MCI Grey matter volume (mm$^3$)</th>
<th>MCI White matter hyperintensity volume (cm$^3$)</th>
<th>AD Grey matter volume (mm$^3$)</th>
<th>AD White matter hyperintensity volume (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI) P</td>
<td>OR (95%CI) P</td>
<td>OR (95%CI) P</td>
<td>OR (95%CI) P</td>
</tr>
<tr>
<td>Delusions</td>
<td>0.393 (0.121 - 1.163) 0.101</td>
<td>0.912 (0.633 - 1.074) 0.500</td>
<td>N/A N/A</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0.916 (0.242 - 3.351) 0.895</td>
<td>0.995 (0.736 - 1.121) 0.960</td>
<td>N/A N/A</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>0.840 (0.546 - 1.284) 0.423</td>
<td>1.001 (0.938 - 1.053) 0.965</td>
<td>1.712 (1.005 - 3.007) 0.053</td>
<td>0.984 (0.894 - 1.071) 0.719</td>
</tr>
<tr>
<td>Depression</td>
<td>1.110 (0.784 - 1.574) 0.555</td>
<td>0.985 (0.928 - 1.034) 0.579</td>
<td>1.075 (0.654 - 1.781) 0.775</td>
<td>1.086 (1.000 - 1.182) 0.048*</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.982 (0.629 - 1.530) 0.936</td>
<td>1.004 (0.936 - 1.060) 0.892</td>
<td>1.235 (0.724 - 2.134) 0.440</td>
<td>1.051 (0.958 - 1.147) 0.267</td>
</tr>
<tr>
<td>Euphoria</td>
<td>0.386 (0.128 - 1.067) 0.075</td>
<td>0.972 (0.764 - 1.084) 0.736</td>
<td>N/A N/A</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Apathy</td>
<td>0.674 (0.436 - 1.030) 0.071</td>
<td>0.930 (0.838 - 1.006) 0.122</td>
<td>0.917 (0.560 - 1.493) 0.726</td>
<td>1.059 (0.978 - 1.147) 0.156</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.784 (0.450 - 1.350) 0.383</td>
<td>0.984 (0.887 - 1.055) 0.710</td>
<td>1.500 (0.809 - 2.868) 0.204</td>
<td>1.095 (0.999 - 1.199) 0.047*</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>0.785 (0.532 - 1.149) 0.215</td>
<td>1.052 (1.007 - 1.101) 0.022*</td>
<td>0.918 (0.547 - 1.532) 0.743</td>
<td>1.054 (0.968 - 1.146) 0.212</td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>0.865 (0.388 - 1.894) 0.718</td>
<td>0.935 (0.744 - 1.060) 0.458</td>
<td>1.227 (0.631 - 2.412) 0.546</td>
<td>1.072 (0.971 - 1.176) 0.147</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>0.979 (0.651 - 1.471) 0.920</td>
<td>0.963 (0.884 - 1.026) 0.317</td>
<td>0.894 (0.464 - 1.703) 0.733</td>
<td>1.116 (1.016 - 1.228) 0.022*</td>
</tr>
<tr>
<td>Appetite change</td>
<td>0.795 (0.472 - 1.324) 0.381</td>
<td>0.974 (0.881 - 1.045) 0.551</td>
<td>0.728 (0.389 - 1.325) 0.304</td>
<td>1.084 (0.991 - 1.184) 0.070</td>
</tr>
</tbody>
</table>

OR=odds ratio; CI=confidence Interval
*Significant results, p<0.05
Table 9. Ordinal logistic regressions for contribution of temporal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects separately.

<table>
<thead>
<tr>
<th>NPI Symptom</th>
<th>MCI Grey matter volume (mm$^3$)</th>
<th>MCI White matter hyperintensity volume (cm$^3$)</th>
<th>AD Grey matter volume (mm$^3$)</th>
<th>AD White matter hyperintensity volume (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P</td>
<td>OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>Delusions</td>
<td>1.245 (3.269e-01 - 5.264)</td>
<td>0.756</td>
<td>0.0001 (2.218e-12 - 0.303)</td>
<td>0.145</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0.528 (0.118 - 2.571)</td>
<td>0.413</td>
<td>0.979 (0.063 - 3.357)</td>
<td>0.981</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>0.950 (0.571 - 1.601)</td>
<td>0.847</td>
<td>1.356 (0.785 - 2.198)</td>
<td>0.235</td>
</tr>
<tr>
<td>Depression</td>
<td>1.139 (0.738 - 1.766)</td>
<td>0.559</td>
<td>0.946 (0.554 - 1.506)</td>
<td>0.824</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.863 (0.506 - 1.484)</td>
<td>0.590</td>
<td>0.665 (0.269 - 1.308)</td>
<td>0.306</td>
</tr>
<tr>
<td>Euphoria</td>
<td>0.359 (0.114 - 1.160)</td>
<td>0.0802</td>
<td>1.329 (0.310 - 3.199)</td>
<td>0.606</td>
</tr>
<tr>
<td>Apathy</td>
<td>0.802 (0.476 - 1.351)</td>
<td>0.407</td>
<td>1.358 (0.778 - 2.234)</td>
<td>0.244</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.769 (0.390 - 1.520)</td>
<td>0.447</td>
<td>1.119 (0.502 - 2.059)</td>
<td>0.746</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>0.823 (0.518 - 1.306)</td>
<td>0.406</td>
<td>1.138 (0.661 - 1.839)</td>
<td>0.613</td>
</tr>
<tr>
<td>Aberrant Motor Behaviour</td>
<td>1.123 (0.414 - 3.104)</td>
<td>0.822</td>
<td>1.963 (0.859 - 3.850)</td>
<td>0.063</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>1.028 (0.623 - 1.711)</td>
<td>0.913</td>
<td>1.297 (0.750 - 2.115)</td>
<td>0.314</td>
</tr>
<tr>
<td>Appetite change</td>
<td>0.782 (0.423 - 1.458)</td>
<td>0.435</td>
<td>0.520 (0.151 - 1.226)</td>
<td>0.215</td>
</tr>
</tbody>
</table>

OR=odds ratio; CI=confidence Interval
*p<0.05

*Significant results, p<0.05
Table 10. Ordinal logistic regressions for contribution of parietal lobe grey matter and WMH volumes to the presence of NPI symptoms in MCI (n=280) and AD (n=99) subjects separately.

<table>
<thead>
<tr>
<th>NPI Symptom</th>
<th>MCI Grey matter volume (mm³)</th>
<th>MCI White matter hyperintensity volume (cm³)</th>
<th>AD Grey matter volume (mm³)</th>
<th>AD White matter hyperintensity volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delusions</td>
<td>1.526 (0.419 - 5.616)</td>
<td>0.520 (0.086 - 1.129)</td>
<td>0.231 N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0.661 (0.112 - 3.361)</td>
<td>0.629 (0.656 - 1.649)</td>
<td>0.457 N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>1.064 (0.628 - 1.797)</td>
<td>0.817 (0.743 - 1.147)</td>
<td>0.606 1.977 (1.089 - 3.788)</td>
<td>0.031* (0.652 - 1.146)</td>
</tr>
<tr>
<td>Depression</td>
<td>1.259 (0.821 - 1.938)</td>
<td>0.291 (0.817 - 1.142)</td>
<td>0.783 1.117 (0.658 - 1.916)</td>
<td>0.682 1.062 (0.841 - 1.339)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.960 (0.553 - 1.656)</td>
<td>0.883 (0.714 - 1.131)</td>
<td>0.495 1.235 (0.724 - 2.134)</td>
<td>0.440 1.051 (0.958 - 1.147)</td>
</tr>
<tr>
<td>Euphoria</td>
<td>0.468 (0.118 - 1.652)</td>
<td>0.254 (0.714 - 1.508)</td>
<td>0.510 N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Apathy</td>
<td>0.763 (0.451 - 1.274)</td>
<td>0.305 (0.622 - 1.044)</td>
<td>0.160 0.908 (0.549 - 1.501)</td>
<td>0.706 1.061 (0.842 - 1.341)</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.825 (0.415 - 1.613)</td>
<td>0.577 (0.735 - 1.235)</td>
<td>0.928 1.522 (0.791 - 3.080)</td>
<td>0.221 1.012 (0.737 - 1.312)</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>0.950 (0.593 - 1.513)</td>
<td>0.828 (0.918 - 1.254)</td>
<td>0.324 0.962 (0.554 - 1.679)</td>
<td>0.892 0.998 (0.761 - 1.272)</td>
</tr>
<tr>
<td>Aberrant Motor</td>
<td>0.913 (0.340 - 2.374)</td>
<td>0.854 (0.415 - 1.211)</td>
<td>0.425 1.004 (0.498 - 2.065)</td>
<td>0.990 1.170 (0.868 - 1.549)</td>
</tr>
<tr>
<td>Behaviour</td>
<td>1.061 (0.643 - 1.743)</td>
<td>0.816 (0.673 - 1.071)</td>
<td>0.238 1.017 (0.526 - 2.008)</td>
<td>0.960 1.105 (0.822 - 1.443)</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>0.893 (0.472 - 1.664)</td>
<td>0.723 (0.751 - 1.220)</td>
<td>0.912 0.561 (0.290 - 1.054)</td>
<td>0.076 1.132 (0.848 - 1.481)</td>
</tr>
<tr>
<td>Appetite change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR=odds ratio; CI=confidence Interval
*Significant results, p<0.05
3.4 WMH and GM volume as predictors of NPS development in MCI and AD

In the current study, 118 subjects had complete longitudinal data, consisting of GM volumes, WMH volumes, and NPI assessment data, at baseline, 12-month and 24-month visits. While 101 subjects did not change, 11 subjects progressed in their diagnosis from MCI to AD between baseline and 24 months. Six subjects converted from MCI to normal diagnosis and were removed from analysis. The final longitudinal dataset consisted of 112 subjects.

Demographic details are summarized in Table 11. Of the 112 participants examined over a 2-year period, 10 were diagnosed as AD and 102 were MCI. AD subjects were significantly older than MCI participants (p=0.032). On baseline cognitive assessment, AD participants performed worse on the Mini Mental State Exam (MMSE) and had worse scores on the CDRSB. AD subjects also had poorer overall scores for symptom frequency and severity on the NPI. MCI and AD subjects did not differ significantly in sex or education level.

3.4.1 Longitudinal change in GM volume

Paired samples t-tests were used to compare mean frontal, temporal and parietal GM volume at baseline, 12 months and 24 months. In the frontal lobe, there was a statistically significant decrease in GM volume between baseline and 12 months (t(111) = 3.437, p = 0.001) but frontal GM volume did not decrease significantly between 12 months and 24 months (t(111) = 1.968, p=0.052). In the temporal lobe, there was a statistically significant decrease in GM volume between baseline and 12 months (t(111) = 5.513, p < 0.001) and 12 months and 24 months (t(111) = 3.793, p < 0.001). Similarly, in the parietal lobe, there was a statistically
significant decrease in GM volume between baseline and 12 months \((t(111) = 2.780, \ p= 0.006)\) and 12 months and 24 months \((t(111) = 3.071, \ p = 0.003)\).

Between baseline and 24 months, frontal lobe GM volume decreased significantly by 0.158mm\(^3\), temporal lobe GM volume decreased significantly by 0.176mm\(^3\) and parietal lobe GM volume decreased significantly by 0.143mm\(^3\).

### 3.4.2 Longitudinal change in WMH volume

Wilcoxon signed-rank tests determined that there was a statistically significant median increase in WMH volume of the frontal lobe between baseline and 24 months \((z = 4.149, \ p < 0.001)\) and between 12 months and 24 months \((z = 4.887, \ p < 0.001)\) but did not increase significantly between baseline and 12 months \((z = 1.401, \ p = 0.161)\). Results also showed a significant increase in WMH volume of the parietal lobe between baseline and 24 months \((z = 2.945, \ p < 0.001)\) and between 12 months and 24 months \((z = 2.738, \ p < 0.001)\) but not between baseline and 12 months \((z = 0.288, \ p = 0.773)\). Temporal lobe WMH volume did not increase significantly over time.

Between baseline and 24 months, frontal lobe WMH volume increased significantly by 0.650cm\(^3\) and parietal lobe WMH volume increased significantly by 0.168cm\(^3\).

### 3.4.3 GM and WMH as predictors of NPS

NPS were combined into psychosis (delusions, hallucinations, sleep), apathy (apathy, appetite), hyperactivity (agitation, euphoria, disinhibition, irritability, aberrant motor behaviour) and affective (depression, anxiety) subsyndromes based on Aalten et al. (2007). A generalized linear mixed model examined the contribution of GM and WMH volume to the presence of these
symptom clusters over a 2-year period. We found the apathy subsyndrome to be associated with decreased GM volume in the temporal \( (z = -2.873, p=0.004) \) and parietal \( (z = -2.531, p=0.011) \) lobes. We also found GM atrophy to be associated with the hyperactivity subsyndrome in the parietal lobe \( (z = -2.125, p=0.034) \).

Table 11. Demographic information at baseline for longitudinal analysis of MCI and AD subjects.

<table>
<thead>
<tr>
<th></th>
<th>MCI (n=102), mean (SD)</th>
<th>AD (n=10), mean (SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70.3 (6.9)</td>
<td>75.3 (8.1)</td>
<td>0.032*</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>61/41</td>
<td>5/5</td>
<td>0.548</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.6 (2.6)</td>
<td>14.9 (2.9)</td>
<td>0.053</td>
</tr>
<tr>
<td>MMSE score †</td>
<td>28.0 (1.6)</td>
<td>22.7 (1.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NPI score †</td>
<td>3.7 (6.1)</td>
<td>9.2 (12.9)</td>
<td>0.041*</td>
</tr>
<tr>
<td>CDRSB score †</td>
<td>1.5 (1.0)</td>
<td>4.9 (2.1)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

MCI=Mild cognitive impairment; AD=Alzheimer’s disease; NPI=Neuropsychiatric Inventory; MMSE=Mini-mental State Exam; CDRSB=Clinical Dementia Rating Scale Sum of Boxes Score
† Scores at baseline
* p<0.05
Table 12. Frequency of neuropsychiatric symptoms (NPS) in subjects at baseline, 12 months and 24 months (n=112).

<table>
<thead>
<tr>
<th>NPI symptom</th>
<th>Baseline (n=112) N (% at baseline)</th>
<th>12 months (n=112) N (% at 12 months)</th>
<th>24 months (n=112) N (% at 24 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delusions</td>
<td>1 (0.9)</td>
<td>4 (3.6)</td>
<td>8 (7.1)</td>
</tr>
<tr>
<td></td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>1 (0.9)</td>
<td>2 (1.8)</td>
<td>7 (6.3)</td>
</tr>
<tr>
<td></td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
</tr>
<tr>
<td>Agitation/Aggression</td>
<td>14 (12.5)</td>
<td>17 (15.2)</td>
<td>28 (25.0)</td>
</tr>
<tr>
<td></td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
<td>3 (2.7) on neuroleptics</td>
</tr>
<tr>
<td>Depression</td>
<td>24 (21.4)</td>
<td>22 (19.6)</td>
<td>30 (26.8)</td>
</tr>
<tr>
<td></td>
<td>43 (38.4) on antidepressants</td>
<td>47 (42.0) on antidepressants</td>
<td>51 (45.5) on antidepressants</td>
</tr>
<tr>
<td>Anxiety</td>
<td>18 (16.1)</td>
<td>16 (14.3)</td>
<td>18 (16.1)</td>
</tr>
<tr>
<td></td>
<td>11 (9.8) on anxiolytics</td>
<td>13 (11.6) on anxiolytics</td>
<td>14 (12.5) on anxiolytics</td>
</tr>
<tr>
<td>Apathy</td>
<td>18 (16.1)</td>
<td>24 (21.4)</td>
<td>26 (23.2)</td>
</tr>
<tr>
<td></td>
<td>43 (38.4) on antidepressants/stimulants</td>
<td>47 (42.0) on antidepressants/stimulants</td>
<td>51 (45.5) on antidepressants/stimulants</td>
</tr>
<tr>
<td>Euphoria</td>
<td>2 (1.8)</td>
<td>1 (0.9)</td>
<td>5 (4.5)</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>11 (9.8)</td>
<td>9 (8.0)</td>
<td>9 (8.0)</td>
</tr>
<tr>
<td>Irritability/Lability</td>
<td>30 (26.8)</td>
<td>28 (25.0)</td>
<td>34 (30.4)</td>
</tr>
<tr>
<td>Aberrant motor behaviour</td>
<td>6 (5.4)</td>
<td>5 (4.5)</td>
<td>14 (12.5)</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>18 (16.1)</td>
<td>24 (21.4)</td>
<td>25 (22.3)</td>
</tr>
<tr>
<td>Appetite change</td>
<td>12 (10.7)</td>
<td>12 (10.7)</td>
<td>18 (16.1)</td>
</tr>
</tbody>
</table>

NPI=Neuropsychiatric Inventory; AD=Alzheimer’s Disease; MCI=mild cognitive impairment
Significant results, *p<0.05, **p<0.01, ***p<0.001
Table 13. Frequency of neuropsychiatric symptom (NPS) subsyndromes in subjects at baseline, 12 months and 24 months (n=112).

<table>
<thead>
<tr>
<th>NPS subsyndrome</th>
<th>Baseline (n=112)</th>
<th>12 months (n=112)</th>
<th>24 months (n=112)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (% at baseline)</td>
<td>N (% at 12 months)</td>
<td>N (% at 24 months)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>18 (16.1)</td>
<td>27 (24.1)</td>
<td>31 (27.7)</td>
</tr>
<tr>
<td>Apathy</td>
<td>23 (20.5)</td>
<td>30 (26.8)</td>
<td>32 (28.6)</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>42 (37.5)</td>
<td>40 (35.7)</td>
<td>52 (46.4)</td>
</tr>
<tr>
<td>Affective</td>
<td>32 (28.6)</td>
<td>32 (28.6)</td>
<td>39 (34.8)</td>
</tr>
</tbody>
</table>
Table 14. Summary of generalized linear mixed model results for the frontal, temporal and parietal lobes in MCI and AD, combined (n=112).

<table>
<thead>
<tr>
<th>NPS Subsyndrome</th>
<th>Frontal Lobe</th>
<th>Temporal Lobe</th>
<th>Parietal Lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grey matter volume (mm$^3$)</td>
<td>White matter hyperintensity volume (cm$^3$)</td>
<td>Grey matter volume (mm$^3$)</td>
</tr>
<tr>
<td></td>
<td>$z$</td>
<td>$P$</td>
<td>$z$</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.546</td>
<td>0.585</td>
<td>1.504</td>
</tr>
<tr>
<td>Apathy</td>
<td>-1.045</td>
<td>0.296</td>
<td>1.316</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>-1.053</td>
<td>0.293</td>
<td>0.720</td>
</tr>
<tr>
<td>Affective</td>
<td>0.013</td>
<td>0.990</td>
<td>-0.440</td>
</tr>
</tbody>
</table>

NPS=Neuropsychiatric symptoms
Significant results, *p<0.05, **p<0.01
Chapter 4  Discussion

In this study, we evaluated the contribution of GM and WMH volume to neuropsychiatric symptoms in patients with MCI or AD. We examined subjects with MCI and AD, and aimed to (1) assess the relationship between WMH and GM volumes to the presence of NPS and (2) assess the relative contribution of WMH and GM volumes to the development of neuropsychiatric symptom over time. We have shown that GM atrophy and increased WMH volume in specific lobes of the brain are associated with specific NPS. Moreover, we identified brain regions where GM and WMH volume predicted the progression of NPS subsyndromes. To our knowledge, this study is the first to examine the trajectories of GM and WMH volumes over time and their relative contributions to NPS impairment in MCI and AD subjects.

4.1 NPS frequency in MCI and AD

In the current study, the most frequent NPI symptom observed among AD subjects was apathy, followed by depression, irritability and agitation. These findings are similar to those in the literature on NPS in other MCI, AD and dementia populations. For instance, Lyketsos and colleagues (2002) found depression, apathy and anxiety to be the most frequently reported symptoms in patients with dementia in a large population-based study. Likewise, meta-analyses of the literature on NPS in AD (Boublay et al., 2016; Zhao et al., 2016) identified apathy and depression to be the two most frequent neuropsychiatric symptoms in this population.

The frequency of NPS in MCI subjects in this study are also comparable to those reported by other groups. The most frequent NPI symptom reported by individuals with MCI in the current population was depression, followed by irritability, sleep disturbance and apathy.
Apathy, irritability and depression were the 3 most frequent symptoms observed among patients with MCI in studies by Hwang et al. (2004), Geda et al. (2009) and Lyketsos et al. (2002).

Patients can experience more than one neuropsychiatric symptom (Lyketsos et al., 2002; Starkstein et al., 2001). Lyketsos et al. (2002) found that 55% of dementia patients reported 2 or more neuropsychiatric symptoms while 44% of patients reported 3 or more. Up to 50% of individuals with MCI have been reported to experience at least a single NPS since cognitive symptom onset (Lyketsos et al., 2002). In the current study, 84% (83/99) of AD subjects had at least one NPS, and 58% (162/280) of MCI had at least a single NPS. 64% (63/99) of AD had more than one symptom while 34% (94/280) of MCI had more than one symptom. In the current study, we also considered medication use in MCI and AD subjects and ascribed positive NPI for specific NPS (delusions, hallucinations, agitation/depression, anxiety and apathy) if on medications targeting these symptoms. Even greater frequency of symptoms are reported when including subjects on medication: 92% (91/99) of AD subjects and 71% (198/280) of MCI had at least a single NPS, while 80% (79/99) of AD and 57% (159/280) of MCI had more than one symptom. In AD, higher total NPI score was associated with greater temporal lobe (r=0.168, p=0.048) WMH burden. GM and WMH volumes did not correlate with the total number of NPI symptoms or overall NPI score in other lobes for MCI and AD subjects. However, we found significant relationships between GM and WMH volumes and specific NPI symptoms.

The proportion of subjects with NPS was found to be greater in subjects with AD than MCI in both the current study and in previous studies of NPS in AD and MCI (Lopez et al., 2005; Van der Mussele et al., 2013). Depression is one of the most frequently reported symptoms in both MCI and AD groups, however it was reported in 41.4% of AD subjects in this study compared to 25.7% of those with MCI. All the NPS except for euphoria and sleep disturbances,
which were equally likely in both groups, were more frequently observed in AD. This difference has been reported in previous work; for example, Lopez et al. (2005) noted greater symptoms of delusions, hallucinations, and psychomotor agitation in subjects with probable AD compared to MCI, although they did not find differences for major depression or aggression between groups. In the current study, depression and aggression were significantly different in frequency between MCI and AD subjects. This may be because different assessments were used to measure symptoms. In the study by Lopez and colleagues (2005), depression was measured using the Hamilton Depression Rating Scale and aggression was measured using evidence of verbal or physical aggressive behaviour. The current study used the NPI, which measures symptoms using an informant-based interview. Moreover, medication use was also considered in this study.

The frequency and severity of symptoms vary for different NPS. Similar to previous findings (Aalten et al., 2007), psychotic symptoms which include delusions and hallucinations were rare in the MCI and AD populations in the current study but showed high NPI scores. Although they appear less frequently, psychotic NPS can have significant and debilitating effects on patient well-being and caregiver burden and would expectedly be rated as having high severity by an informant on the NPI. Psychosis has been frequently associated with increased dependence on caregivers as well as cognitive decline (Zahodne et al., 2015; Nagata et al., 2016). One study clustered NPI symptoms into psychotic/behavioural, depressive and minimally symptomatic groups and found caregiver burden to be highest in the psychotic/behavioural cluster (Rocca et al., 2010). Consequently, although NPS may vary in prevalence, specific symptoms can have a considerable effect on caregiver burden and patient well-being.

Studies on NPS appear to show significant heterogeneity in the prevalence of symptoms. For instance, Zhao et al. (2016) conducted a review and meta-analysis of 48 studies on the
prevalence of NPS in AD and found significant variation in the prevalence of NPS reported across studies. This may be due to a number of factors including the setting and population being studied, the assessment used to evaluate NPS, and the duration of disease examined in a particular study. Many behavioural symptoms may also result from multiple factors such as differences in neuropathology, comorbidities and the physical and social environment (Gitlin et al., 2014). As with the ADNI cohort used in this study, participants in clinical trials are selected based on specific inclusion and exclusion criteria and may not accurately reflect the general population. These factors must be taken into consideration when interpreting the results of the current study.

Finally, our findings of specific NPS in MCI subjects suggest that behaviour impairment can occur years before clinical AD diagnosis. Identifying the behavioural profiles of MCI and AD can help to differentiate between stages of AD. Behavioural symptoms in MCI may also have prognostic value.

4.1.1 NPS progression in MCI and AD

Our data suggest that certain symptoms appear to increase over time, including delusions and hallucinations. For example, in the current study, delusions were 0.9% at baseline, 3.6% at 12 months, and 7.1% at 24 months. This was not found to be true for all symptoms; anxiety and disinhibition did not appear to change noticeably over the 2-year period. Changes in NPS over time are variable and different symptoms have been found to have different trajectories that may increase, decrease or remain stable over time (David et al., 2016; Levi et al., 1996). A systematic review of the literature in dementia found apathy to be the only symptom with high baseline prevalence, persistence and incidence throughout the course of dementia (van der Linde et al., 2016). Further, in a group of 514 dementia patients, Brodaty et al. (2015) found delusions,
hallucinations, agitation, anxiety, apathy, disinhibition, irritability and aberrant motor behaviour to increase over a 3-year period while depression, euphoria, nighttime behaviour and appetite did not significantly increase. In the current study, anxiety and disinhibition did not appear to change noticeably over time. However, different dementias have been shown to have unique neuropsychiatric features (Hirono et al., 1999) which may explain these differences in NPS development compared to our study population of MCI and AD subjects.

It is also possible that some symptoms will develop at later stages of disease. Zhao et al. (2016) had found delusions to occur more frequently in older subjects. Similarly, Levi et al. (1996) found that patients in their oldest age group (76-87 years) had more psychosis and less depression and agitation. The current study found psychotic symptoms (delusions and hallucinations) to increase over time, however we did not examine changes in symptoms with age.

Mild behavioural impairment was proposed as a syndrome characterized by behavioural changes and mild psychiatric symptoms but without serious cognitive complaints (Taragano et al., 2009). This concept was developed based on studies showing that NPS in cognitively normal older adults increased the risk of developing MCI, as well as other work that described NPS as an important factor in the conversion from MCI to dementia (Geda et al., 2014; Kantarci et al., 2013; Donovan et al., 2014). In this study, we found a relationship between GM atrophy and the progression of apathy and hyperactivity symptoms over time. These results indicate that disease progression may be associated with worsening NPS. However, we did not examine progression from MCI to AD or the relationship between GM or WMH volumes and cognitive decline in these subjects. Future work should compare subjects who progress from MCI to AD with subjects who do not progress in their diagnosis, in order to assess whether NPS may contribute to
the conversion from MCI to AD in the current study population. Moreover, it is important to distinguish whether specific NPS are involved in AD as risk factors or early manifestations of the disease, or whether they are epiphenomenologically associated with AD. For instance, depressive symptoms often occur early in AD pathogenesis, even before diagnosis, and may act as an early indicator of disease. Further research is needed to be able to elucidate how specific NPS are involved in AD pathogenesis.

4.1.2 Medication use

The majority of AD patients suffer from NPS, and many use medications to treat their behavioural symptoms. Subject medication use was included in the current study to incorporate those who experience NPS but may not display symptoms due to their medication use, including neuroleptics, antidepressants, stimulants and anxiolytics. These subjects would not be considered symptomatic on the NPI and may be overlooked when assessing NPS. Clinical trials have examined the efficacy of various medications on the treatment of specific NPS. Although results have been mixed, a number of studies show modest improvements in symptoms (Holmes et al., 2004; Hermann et al., 2011; Small & Bullock., 2011). For instance, ChEIs may ameliorate apathy and mood disturbances, and memantine may improve agitation and irritability (Gauthier et al., 2010).

4.2 Identifying regions of interest associated with NPS

Deformation-based morphometry was carried out to identify regions of interest across the brain associated with individual NPI symptoms without specific a priori hypotheses, in MCI and AD subjects. We did not find any brain regions to be significantly associated with NPI symptoms after a false discovery rate for multiple comparisons (q=0.05). One explanation for our results
may be that GM and WM have different contributions to these symptoms as DBM uses a whole-brain approach that does not segment tissue type. While this technique is valuable because it avoids bias towards any particular structure or tissue (Ashburner et al., 1998), lack of separation of tissue type may combine GM and WM where these could have distinct relationships with NPS. Moreover, it is possible that some regions will have been missed as a result of multiple comparisons correction.

4.3 Contribution of GM and WMH volume to NPS

4.3.1 Contribution of WMH volume to NPS

Our results showed greater WMH volume to be consistently associated with higher symptoms of irritability, especially among patients with MCI. Subjects with high WMH load were more likely to show irritability. Moreover, in MCI and AD, higher WMH volume in the frontal lobe was related to greater irritability as measured on NPI. When MCI and AD were examined separately, only MCI continued to show this association. When combining the MCI and AD group, higher depression, disinhibition and sleep were associated with greater WMH volume in the frontal lobe.

Irritability and depression and were among the three most common symptoms in MCI and AD subjects in the current study and have been reported to be high in MCI and AD populations in other studies (Lyketsos et al., 2002). Similarly, affective dysregulation, which included depression and irritability symptoms, was shown to be the most prevalent among a group of symptom clusters or subsyndromes that included social inappropriateness, impulse dyscontrol, abnormal perception and decreased motivation (Ismail et al., 2016).
Notably, neuropsychiatric symptoms in this study were associated with WMH volume in the frontal lobe. Previous work on metabolic dysfunction using $^{18}$F-FDG-PET has found hyperactivity and affective scores to be associated with increased glucose metabolism in the frontal and limbic structures (Ballarini et al., 2016). Whole brain analysis identified regions within the medial right frontal lobe as important for regulating behaviours measured by the NPI (Rosen et al., 2005). Difficulty with regulating behaviour may contribute to symptoms of disinhibition. In subjects with traumatic brain injury, significantly reduced left prefrontal grey matter volumes, particularly in the ventrolateral and dorsolateral regions, have been associated with major depression (Jorge et al., 2004). Animal studies have also found correlates of depression in the frontal lobe; changes in gene expression in the frontal cortex have been related to depressive-like symptoms in mice (Tordera et al., 2011). Previous work has also found that most WMH associated with CVD are located in the frontal lobe (Mortamais et al., 2013).

No symptom cluster or subsyndrome was found to be associated with WMH volume in our longitudinal analysis. By combining symptoms into subsyndromes, we may have masked more specific relationships with NPS, such as the association between irritability and WMH volume observed in our other analyses.

### 4.3.1.1 Cerebrovascular disease implications

The vascular depression hypothesis by Alexopolous et al. (1997) proposes a relationship between depression and CVD in older adults. Our findings of higher WMH volume associated with depression provide support for the relationship between depression and CVD, although we did not find that WMH volume contributed to depression over time. A similar study on AD subjects looked at WMH volumes of the frontal, temporal, parietal and occipital lobes and their relationship with depressive disorders (minor and major depressive disorder, dysthmic disorder
and subsyndromal depression) (Lee et al., 2015). They also found higher frontal lobe white matter lesion volume to be significantly associated with depression. CVD may be involved in depression through molecular mechanisms including endothelial dysfunction and inflammation (Santos et al., 2012). Post-mortem studies have identified greater levels of proinflammatory factors in the dorsolateral prefrontal cortex in individuals with late life depression (Thomas et al., 2002). This has also been observed in vivo (Dimopoulos et al., 2006). In addition to CVD, various other mechanisms have been proposed to explain the relationship between white matter deficits and depression including decreased oligodendrocyte density and reduced gene expression in the prefrontal cortex (Tham et al 2011).

4.3.2 Contribution of GM volume to NPS

Lower GM volume in the frontal, temporal and parietal lobes was significantly associated with apathy, appetite change and hallucinations. Moreover, GM atrophy in the parietal and temporal lobes predicted apathy and appetite change symptoms over 2 years (apathy subsyndrome). These results are similar to findings of apathy and appetite change associated with lower GM volume in our cross-sectional analysis.

Apathy is highly prevalent in both MCI and AD subjects and some studies have shown apathy to be associated with AD progression and functional impairment (Palmer et al., 2010; Wadsworth et al., 2012). Apathy has frequently been associated with structural and functional deficits in the brain. For example, apathy has been related to reduced perfusion in the anterior temporal, orbitofrontal, anterior cingulate and dorsolateral prefrontal regions (Craig et al., 1996). Other studies have found apathy to be associated with frontal brain regions (Bruen et al., 2008; Hahn et al., 2013; Tunnard et al., 2011). Hyperactivity and affective scores were associated with increased glucose metabolism in the frontal and limbic structures while apathy scores were
negatively correlated with bilateral orbitofrontal and dorsolateral frontal cortex metabolism (Bellarini et al., 2016). Moreover, apathy has also been associated with poorer functional connectivity in the frontoparietal control network (Munro et al., 2015). The role of the anterior regions of the brain in regulating behaviour suggests that atrophy of structures within the frontoparietal region may lead to deficits in affective and impulse control and may contribute to the symptoms of apathy and appetite change observed in the present study.

Among AD subjects, higher depression, disinhibition and sleep were associated with greater WMH volume in the frontal lobe. Lower temporal lobe GM volume was associated with less disinhibition, and lower GM in the parietal lobe was related to less agitation. Lower disinhibition and agitation may be the result of increased apathy among AD patients. Likewise, we found GM atrophy of the temporal and parietal lobes to be associated with increased apathy over a 2-year period. We also did not find disinhibition to increase in prevalence over time between baseline, 12-month and 24-month visits. It is possible that apathy and disinhibition are opposing NPS such that an increase in one symptom may be associated with a decrease in the other. Recently, O’Connor et al. (2016) found similar results of reduced disinhibition and increased apathy and eating changes in bvFTD patients. Longitudinal studies that compare the relationship between these NPS over time may be able to determine if such an interaction exists and the contribution of GM volume to the development and progression of these symptoms in MCI and AD.

Neuropsychiatric symptoms have been associated with frontotemporal degeneration in other neurodegenerative diseases such as FTD, PD, HD and PSP. Differential symptom prevalence exists between these diseases; for instance, patients with AD, FTD and PSP had more
severe and higher prevalence of apathy and less depression compared to patients with PD and HD (Levy et al., 1998).

4.3.2.1 Neurobiology of NPS in AD

Three neurobiological models have shown relevance to NPS in AD: the frontal-subcortical circuits, the cortico-cortical networks, and the monoaminergic system (Geda et al., 2013). Frontal-subcortical circuits are involved in mediating human behaviour, and dysfunction in these circuits has been associated with impaired executive functions, apathy, and impulsivity (Bonelli et al., 2007). One of the major fronto-subcortical circuits is the anterior cingulate circuit involved in motivated behaviour and apathy. In this study, apathy was associated with lower GM volume of the frontal, temporal and parietal lobes, although only temporal and parietal lobe GM was related to apathy over time. A large component of the anterior cingulate circuit is its connection with subcortical structures, including the limbic striatum and the thalamus, which were not examined in the current study.

A number of mechanisms may explain the relationship between NPS in AD. For instance, one possibility is that underlying neuropathology directly contributes to NPS. It is also possible that the experience of cognitive decline in AD leads to the development of symptoms like depression and apathy. However, this mechanism is unlikely to explain all NPS in AD, including psychosis, and the presence of NPS in other degenerative diseases that are not characterized by cognitive impairment. Genetic and environmental factors may also play a role in NPS development and an interaction among various factors could likely explain the development and progression of NPS in AD.
4.4 Clinical implications

NPS are an important source of patient distress and caregiver burden, and these symptoms are a primary contributor to institutionalization. Medications are frequently used to treat behavioural symptoms in AD and other dementia populations. However, studies examining the efficacy of these medications, including antidepressants and antipsychotics, have not shown consistent results in improvement of behavioural symptoms (Lyketsos et al. 2000; Rosenberg et al. 2012). Moreover, medications can often contribute to adverse affects, and often a combination of treatment options with medications is recommended (Koenig et al., 2016). Further understanding of the pathophysiology of AD may help to improve the efficacy of medications used to treat NPS in these patients.

This study identifies a relationship between WMH volume in certain brain regions with specific NPS. Cerebrovascular disease is a common comorbidity of AD (de la Torre., 2002). Further, cerebrovascular disease, which is largely believed to be the underlying cause of WMH, has a number of modifiable risk factors, i.e. lower blood pressure, control diabetes and hypercholesterolemia. As a result, interventions that are aimed at reducing vascular disease may prove beneficial in treating these symptoms. Identification of focal lesions can also lead to therapeutic trials focused on targeting individuals with similar lesions and to further research examining the specific NPS associated with these regions.

4.5 Limitations

The results in this study were not corrected for multiple comparisons due to the exploratory nature of the analyses. In addition, this study was limited by the exclusion of subjects with psychotic features, agitation or behavioural problems within 3 months prior to
screening that would interfere with ADNI protocol compliance. This exclusion of patients with significant behavioural problems would have reduced the number of patients with neuropsychiatric symptoms and may contribute to the low number of patients with NPI psychosis observed in the current study and in similar studies. These exclusions would also have limited NPS severity in our study population. MRI scans were also collected from different sites and using multiple scanners. We also considered medication use in our study population; however, we cannot be certain that medications were prescribed to treat the specific NPS for which the medication is primarily indicated (i.e. depression present if on an antidepressant) as was assumed for the current study, and off-label prescription of psychiatric medications is common. However, because a large number of AD patients take medications to treat specific NPS, the effect of medication use on NPS cannot be overlooked when identifying symptomatic and asymptomatic subjects.

A limitation in our assessment of WMH volume contribution to NPS is the Hachinski Ischemic Score cutoff of 4 that was part of the selection criteria used by ADNI for study recruitment, where scores greater than 7 suggest vascular involvement. Although we did find a range of WMH volume across brain regions, this criterion likely excluded patients with severe WMH burden and limited findings in the current study.

The current study examines subjects from a multi-site research population who are selected based on specific criteria that may limit interpretation of results concerning the general population. Nevertheless, ADNI has developed standardized protocols that can be used globally to compare results across studies, and future work will be able to better elucidate the extent to which these subjects can be compared to the general population. Moreover, although the NPI has been shown to have established validity and reliability, it is based on caregiver reporting of a
patient’s NPS. Symptoms may be over or under reported and more research is needed to
determine how these reports correspond with clinician observations. A longer observation period
for our longitudinal analysis may also provide a better representation of GM and WMH volume
changes associated with NPS over time. This study also examined subjects with amnestic MCI
and early AD and may not reflect the relationship between volumes and NPS throughout the
progression of AD, including in moderate and severe cases.

4.6 Conclusions

Here we show that greater WMH and lower GM volumes are associated with NPS in
both MCI and AD populations and can act as predictors of symptoms over time. Affective
symptoms, such as depression and apathy, are the most prevalent in the current study population.
We also showed that lower GM volumes contribute to NPS in our combined MCI and AD
population. Greater WMH volume also contributed to symptoms in MCI, AD and combined
MCI and AD subjects, although higher WMH burden appeared to play a more significant role in
contributing to symptoms among AD subjects. Moreover, we show associations between NPS
and GM atrophy of the temporal and parietal lobes.

The results of this study support previous findings of GM volume loss associated with
neuropsychiatric deficits in MCI and AD. These findings also show that GM and WMH volume
relate to different NPS, including irritability associated with WMH volume and affective and
psychotic symptoms associated with GM volume. Our results also suggest a significant
contribution of cerebrovascular disease to NPS in MCI and AD patients and therefore provide
evidence for close monitoring and controlling of vascular risk factors and for promoting a
healthy lifestyle in these patients. Our results and those of previous studies indicate that a
combination of factors, including GM and WMH volume, appear to contribute to NPS in AD and other dementias. The underlying pathogenesis of NPS is likely dependent on the breakdown of multiple brain regions and networks.

Alzheimer’s disease is the most prevalent cause of dementia. As the population continues to grow, along with an increase in the lifespan, the challenges associated with neuropsychiatric symptoms in MCI, AD and all dementias will be of greater concern. Preventative treatments of NPS will become essential for decreasing disease burden. NPS are also common in other dementias, often with distinct neuropsychiatric features. This study could be used to identify the contribution of WMH and regional atrophy to neuropsychiatric symptoms in these diseases.

4.7 Future directions

The results from the current study suggest new directions for future research. One such avenue would be to examine GM and WMH volume changes at a more regional level (i.e. looking specifically at the hippocampus, amygdala, and middle frontal gyrus). Second, while this study examined MCI and early AD subjects, future work that looks at GM and WMH progression based on AD severity at later stages of disease can provide additional information on AD impairment. Finally, a larger period of follow-up with subjects will provide greater clarity of the trajectory of NPS and volumetric changes with disease progression.

4.7.1 Regional segmentation of GM and WMH volume

The current study examined changes in GM lobar volume and WMH volume. These large brain areas encompass many neuroanatomical structures that have been associated with multiple cognitive and behavioural functions. Segmenting GM and WMH at a more regional level may
better identify specific regions where WMH and GM volume changes contribute to NPS development, including examination of subcortical structures. Comparing right and left brain regions would also be valuable as many cognitive and functional deficits tend to be lateralized. Consequently, more precise conclusions could be made about the particular effects of GM and/or WMH damage to NPS.

Many previous studies show specific functional deficits related to particular brain structures. For example, aggression has been associated with the orbitofrontal cortex, amygdala and hippocampus in human and animal studies (Coccaro et al., 2007; Soloff et al., 2003; Machado & Bachevalier 2006). Likewise, if disruption of WM tracts is caused by WMH pathology, examining WMH lesions on a more focal level and in specific anatomic locations would be preferable to a global assessment of WMH burden. Statistical parametric mapping has been used to identify regions containing high WMH volumes associated with symptoms of depression (Sheline et al., 2008; Taylor et al., 2003). WMH brain masks can be used to isolate regions of interest and correlate these changes with NPI symptom scores along with GM regions. Some studies have also compared periventricular and deep WMH volume and found significant differences based on these regions (Park et al., 2011; Meguro et al., 1995; Soennesyn et al., 2012; Starkstein et al., 2009). Moreover, these results could be compared with regions associated with cognitive difficulties in MCI and AD. This would provide a comparison of the relative involvement of these structures with cognitive and behavioural impairment.

4.7.2 NPS and AD severity

Future work could also examine the progression of WMH and GM volume associated with NPS based on AD severity. For example, the Clinical Dementia Rating scale is used to quantify and stage the severity of symptoms in dementia. Determining the effect of WMH and
GM volume to NPS at various stages of AD could help examine this relationship throughout AD progression. These methods can also be applied to other dementias with neuropsychiatric features. Future work should look at differences and changes in NPS at baseline and longitudinally and assess whether the trajectory of NPS and relationship between GM, WMH and NPS differ based on symptom severity. Currently, AD diagnosis is based on cognitive impairment. Incorporation of NPS may provide a more precise and comprehensive diagnosis of AD and specific disease stage.

In addition, an extension of this study would be to examine NPI scores longitudinally, which take into account both frequency and severity of NPS. The current study aimed to examine the relationship between GM and WMH volumes and NPS at baseline and longitudinally, and future work assessing symptom scores could provide additional information on the contribution of both GM and WMH volumes to disease severity over time. Future work could also investigate whether MCI subjects with NPS are at greater risk of progression to AD or other dementias compared to MCI without these symptoms.

4.7.3 Longitudinal studies of NPS progression

There are a limited number of studies that examine neuropsychiatric symptoms in this population, and even fewer looking at longitudinal changes. Moreover, there is a wide variety of MRI techniques and analyses used which make it difficult to perform direct comparisons between studies. Further longitudinal studies that span a longer time course will be instrumental in improving our understanding of how changes in GM and WMH volumes may be associated with NPS throughout the stages of AD and other dementias. Likewise, this knowledge would help to differentiate between healthy aging and the progression towards MCI, AD or other dementias. Finally, such analyses could also help in identifying neuropsychiatric deficits early in
the disease course, which would be beneficial for developing disease-modifying treatments and improving overall quality of life in AD patients.

As AD is increasingly defined based on the measurement of biomarkers, especially amyloid and tau, future work could examine their role in the development and progression of NPS. For instance, the CSF tau to amyloid ratio could be used to measure the progression of these biomarkers compared to NPS progression in MCI and AD subjects. Moreover, the distribution of amyloid and tau can be examined in relation to regions with high WMH volume or grey matter atrophy. Further research is needed to better identify the factors contributing to NPS, their possible interactions and their relative contribution to NPS development and progression.
References


Copyright Acknowledgements

Figure 2. Reprinted from The Lancet Neurology, Vol. 12, C.R. Jack et al., Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers, pp. 207-216. Copyright 2013, with permission from Elsevier.
Appendix A

Figure 9. Correlation coefficient maps of agitation symptoms in MCI and AD subjects.

Deformation-based morphometry maps show regional volume changes associated with NPI agitation scores in MCI and AD subjects. Colours represent correlation coefficient values. Results were not significant after FDR correction (q=0.05).
Figure 10. Correlation coefficient maps of anxiety symptoms in MCI and AD subjects.

Deformation-based morphometry maps show regional volume changes associated with NPI anxiety scores in MCI and AD subjects. Colours represent correlation coefficient values. Results were not significant after FDR correction (q=0.05).
Figure 11. Correlation coefficient maps of depression symptoms in MCI and AD subjects.

Deformation-based morphometry maps show regional volume changes associated with NPI depression scores in MCI and AD subjects. Colours represent correlation coefficient values. Results were not significant after FDR correction (q=0.05).